LEGIBILITY NOTICE

A major purpose of the Technical Information Center is to provide the broadest dissemination possible of information contained in DOE's Research and Development Reports to business, industry, the academic community, and federal, state and local governments.

Although a small portion of this report is not reproducible, it is being made available to expedite the availability of information on the research discussed herein.

1

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

ORNL--6035

DE84 013519

NUTRITIONAL REQUIREMENTS AND CONTAMINANT ANALYSIS OF LABORATORY ANIMAL FEEDS

by

Bimal C. Pal
Robert H. Ross
Chemical Effects Information Center
Information Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

and

Harry A. Milman
Health and Environmental Review Division
Office of Toxic Substances
U.S. Environmental Protection Agency
Washington, DC 20460
Interagency Agreement DW930141-01-1

NOTICE
PORTIONS OF THIS REPORT ARE ILLEGIBLE. It has been reproduced from the best available copy to permit the broadest possible availability.

Date Published - May 1984

Prepared For
OFFICE OF TOXIC SUBSTANCES
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

Prepared by the
OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831
operated by
MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U.S. DEPARTMENT OF ENERGY
under Contract No. DE-AC05-84OR21400

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

This report has been reviewed by the Office of Toxic Substances, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

CONTENTS

Page

LIST OF FIGURES v

LIST OF TABLES vii

ACKNOWLEDGMENTS ix

EXECUTIVE SUMMARY xi

1 INTRODUCTION 1

2 NUTRITIONAL REQUIREMENTS FOR LABORATORY ANIMALS - COMPARISON OF THE DATA PROPOSED IN THE EPA GOOD LABORATORY PRACTICE STANDARDS WITH THOSE OF COMMERCIAL PROCEDURES, NIH, AND AIN 5

3 EVALUATION OF DIFFERENT TYPES AND FORMS OF DIETS 6

3.1 Types of Diets - Advantages and Disadvantages 6

3.1.1 Natural-Ingredient Diets 6
3.1.2 Purified Diets 6
3.1.3 Chemically Defined Diets 6
3.1.4 Open Formula Diets 12
3.1.5 Closed Formula Diets 12
3.1.6 Quasi-Open Formula Diets 12

3.2 Forms of Diets 12

3.3 Autoclaving of Diets 14

3.4 Discussion 15

4 DIETARY INTAKE 17

5 VITAMINS AND MINERALS USED IN ANIMAL DIET FORMULATIONS 18

5.1 Vitamins 18
5.2 Minerals 18

6 INGREDIENTS USED IN LABORATORY ANIMAL DIETS 22

7 CONTAMINANTS AND PRESERVATIVES IN ANIMAL FEEDS 24

7.1 Introduction 24
7.2 Biological Contaminants 24

7.2.1 Estrogens 24
7.2.2 Aflatoxins 26
7.2.3 Tricothecene Toxins 27

7.3 Nitrosamines 27
7.4 Preservatives 30
7.5 Tolerance Limits of Contaminants and Preservatives in Animal Feed 31

7.5.1 Considerations for Establishing Tolerances for Contaminants 36
7.5.2 Suggested Changes in the Tolerance Limits for the Contaminants Listed in the EPA GLP 36

7.5.3 Inconsistencies in EPA and FDA Tolerance Limits for Contaminants 44

8 MONITORING AND ANALYSIS OF CONTAMINANTS IN FEED 45

9 INTERACTION OF DIETARY COMPONENTS 47

9.1 Interplay Among Zn, Se, As, Vitamins, Amino Acids, Fatty Acids, and Phytate 47
9.2 Ca:P Ratio 47
9.3 K:Na Ratio 47
9.4 Enzyme Induction and Inhibition of Neoplasia by Minor Dietary Constituents, Feed Additives, and Contaminants 48
9.4.1 Enzyme Induction by Dietary Constituents 48
9.4.2 Enzyme Induction by Feed Additives 48
9.4.3 Enzyme Induction by Contaminants in Animal Feed ... 48
9.4.4 Inhibition of Neoplasia 49
9.4.5 Monitoring of Induced Enzyme Activity 49
9.4.6 Enzyme Induction and Genetic Differences 49

10 SPECIAL TOPICS 50

10.1 Phytates 50
10.2 Arsenic 50
10.3 Selenium 51

11 ANALYSIS OF PUBLIC COMMENTS ON THE PROPOSED EPA GUIDELINES FOR DIETARY REQUIREMENTS AND CONTAMINANT ANALYSIS 52

11.1 Criticisms of Proposed Guidelines and Related Discussion 52
11.2 Analysis of Specific Comments 53

11.2.1 Shelf Life Restrictions 53
11.2.2 Feed Meal 53
11.2.3 Calcium, Phosphorus, Iron, and Cobalt 53
11.2.4 Vitamins 54
11.2.5 Choline and Inositol 54
11.2.6 Alternative Sources of Minerals 55
11.2.7 Dicalcium Phosphate 55
11.2.8 Fat 55
11.2.9 Ash 55
11.2.10 Air Dry 56
11.2.11 Units of Measurement 56

LITERATURE CITED 57

Appendix A. National Institutes of Health Standard for Nutrient and Chemical Contaminant Analyses of Laboratory Animal Diets 71

Appendix B. Conversion Factors for Selected Vitamins 73

Appendix C. Letter from Edward J. Ballitch 74

Appendix D. Abbreviation List 75

Appendix E. Additional Literature References 76

LIST OF FIGURES

Figure Page

1 Growth curve for rabbit (New Zealand White) 8

2 Growth data for guinea pig (Ft. Detrick Dunkin Hartley) 9

3 Growth curves for fifteen breeds of dog 10

LIST OF TABLES

Table **Page**

1 Approximate Nutrient Composition of EPA, NIH, AIN and Commercial Laboratory Animal Diet Formulations 2

2 Estimated Nutritional Requirements of Laboratory Animals 3

3 Normal Growth Rate of the Hamster, Rat, and Mouse 7

4 Reproduction and Life Span Data on Selected Laboratory Animals 11

5 Concentrations of Selected Nutrient and Toxic Components in Random Samples of Rodent Natural Product Diets 13

6 Vitamins, Minerals, and Animal Diet Formulations 19

7 Trace Minerals Included in the GRAS List 21

8 Ingredients Commonly Used in Laboratory Animal Diets 23

9 Estrogens in Animal Feed 25

10 Fusarium Toxins 28

11 Contaminants and Preservatives in Laboratory Animal Feeds 32

12 Pesticides and Contaminants in Laboratory Animal Feeds 33

13 Pesticides in Laboratory Animal Feeds 34

14 Normal Screening Levels of Detection 37

15 Biologically Effective Concentrations of Selected Organic Feed Contaminants 38

16 Biologically Effective Concentrations of Selected Heavy Metals 39

17 Interaction of Dietary Factors with Toxicants 40

18 Results of Analyses of Laboratory Animal Diets 43

19 References to the Methods of Analysis of Contaminants in Animal Feed and Relevant Statistical Data 46

ACKNOWLEDGMENTS

We have received assistance from many people in the preparation of this document. Dr. Gene Bingham, veterinarian, Biology Division, Oak Ridge National Laboratory, and Dr. Joseph J. Knapka, nutritionist, National Institutes of Health, have generously shared their expertise in this area. We are indebted to Betty J. Ward and Edward J. Ballitch of the Food and Drug Administration for enlightening us on the latest regulatory information. Dr. John C. Chah, Zeigler Bros., and Dr. Charles R. Adams, Hoffmann-La Roche, provided information on the stability of fats and vitamins. Thanks are due to Jon H. Ford, Manager, Ralston Purina Company Plant, Richmond, Indiana, for information on the current manufacturing practices in the laboratory animal feed industry. We have received invaluable advice, comments, and suggestions during the preparation of this document from Dr. Damon C. Shelton, Director, Laboratory of Special Chows Research Department, Ralston Purina Company, St. Louis, Missouri. Dr. Harry A. Milman of the U.S. Environmental Protection Agency provided us with a copy of the public comments on the USEPA Dietary Requirements under the Good Laboratory Practice Standards and general guidance during the preparation of this document. Thanks are due to Drs. Joseph J. Knapka (NIH), Kenneth C. Hayes (Harvard), Paul M. Newberne (MIT), and H. Leroy Shilt (Ralston Purina) for their in-depth reviews of this document. We greatly appreciate the assistance of Janice Pruett and Carolyn Seaborn for extensive literature searching and of Donna Stokes and Frances Littleton for the very demanding task of typing.

EXECUTIVE SUMMARY

The primary objectives of this report are to present information concerning the nutritional requirements of several commonly used laboratory animal species (i.e., mouse, rat, hamster, guinea pig, rabbit, and dog) and to discuss various aspects of the problem of contamination of laboratory animal feeds. In addition, this document discusses the different types of laboratory animal diets (e.g., open vs closed formula), the ingredients used in these diets, the interaction of dietary components, and the public comments received respective to the EPA proposed guidelines for the nutrient composition of laboratory animal diets. Much of the data are presented in tabular form as this method seemed the most appropriate.

The principal focus of the discussion on nutritional requirements of laboratory animals is a comparison of the proposed EPA guidelines for rodent diets with the diet compositions for rodents of selected commercial laboratory animal feed manufacturers and with the recommendations of the National Academy of Sciences (NAS) regarding the nutrient composition of laboratory animal diets. These comparisons revealed the following: (1) commercial feed manufacturers are generally in good agreement with the dietary requirements proposed by EPA for rodents; and (2) the levels of nutrients proposed by EPA and found in the commercial feeds are usually higher than those recommended by the NAS. These higher levels result from nutrient concentrations in commercial diets being formulated to compensate for losses during the manufacture and storage as well as for differences in biological availability of nutrients.

The different types of diets include natural-ingredient, purified, chemically defined, open formula, closed formula, and quasi-open formula. The merits and demerits of the open formula versus closed formula diet are a highly controversial issue.

With respect to ingredients used in laboratory animal diets, all of the ingredients listed in the proposed EPA guidelines are used in the National Institutes of Health (NIH) and in the selected commercial diet formulations reviewed. However, some ingredients are included in the NIH and commercial diet formulations that are not included as approved ingredients in the proposed EPA guidelines (USEPA 1979).

Contaminants in laboratory animal feeds arise from biological as well as nonbiological sources. Biological contaminants originate from microorganisms and are mainly estrogens, aflatoxins, and trichothecene toxins. Nonbiological contaminants are usually pesticide residues, nitrosamines, and heavy metals. The EPA proposed guidelines list tolerances for certain contaminants and preservatives, and commercial feed manufactures list maximum acceptable levels for contaminants. For some contaminants (e.g., lindane and cadmium), the maximum allowable levels in commercially manufactured feeds exceed the EPA proposed tolerance levels. No preservatives are allowed according to the proposed EPA

guidelines whereas, for all the commercial producers whose data were reviewed, the preservative butylated hydroxyanisole is used in diets containing animal fats and the preservative ethoxyquin is used to prevent oxidation of vitamin A in diet formulations.

Just as the presence of contaminants in the feed can affect the outcome of a long-term toxicity study, the interaction of dietary components can also play a determining role on the results of a chronic toxicity study. For example, selenium possesses anticarcinogenic properties in animals and humans, and the uptake of selenium can be diminished by zinc (Schrauzer 1977). Knowledge of such interactions would obviously be important for an investigator.

Several commenters (comments regarding the proposed EPA guidelines) disagreed with the guidelines for nutrient composition of laboratory animal diets on the following grounds: (1) lack of feasibility; (2) practicality; (3) scientific justification; (4) lack of harmony with the Food and Drug Administration and the Department of Agriculture; (5) lack of incentive to continue research on diet development; and (6) failure to promote standardization of the diet.

In conclusion, it should be noted that virtually no information exists with respect to the nutrient requirements of laboratory animals beyond the growth and reproductive segments of their life spans. Therefore, it is not known if the nutrient levels proposed by EPA and those in commercial diet formulations are the optimum for a lifetime bioassay study. Research in this area is obviously needed. In addition, the proposed EPA guidelines for diet composition are stated as the recommendations for rodents. However, some confusion arises since no dietary requirement is proposed for vitamin C which is needed in the diet of guinea pigs. Further clarification, or possibly diets recommended for individual species, would obviate this confusion.

1. INTRODUCTION

The coverage in this treatise has been restricted to six laboratory animal species: mouse, hamster, rat, guinea pig, rabbit, and dog. The purpose of this document is to provide the EPA with relevant facts and figures to assist in the revision of the Appendix B, Dietary Requirements and Contaminant Analysis, of the Proposed Good Laboratory Practice Standards for Health Effects (USEPA 1979). Discussion and literature coverage of the nutrient requirements of different laboratory animals has been brief since this information is already available in the excellent monographs published by the National Academy of Sciences (NAS 1974, 1977, 1978). However, Appendix D contains a list of references not cited in this report but pertinent to the area of nutritional requirements of laboratory animals; many of these are cited in the NAS publications.

The manufacture of the natural-ingredient diet, which is the most widely used animal diet, is quite involved and requires an expensive outlay. This is why almost all laboratories depend on the feed manufacturer to provide an adequate and nutritious diet for the animals. Also, these commercial diets have long historical backgrounds and reliability. Representative data on the composition of animal feed from two of the more prominent producers have been collected and presented, along with data on diet composition from the National Institutes of Health (NIH) and the American Institute of Nutrition (AIN) (see Table 1), to provide the basis for comparison with the proposed EPA guidelines (also included in Table 1) and discussion of the state-of-the-art of dietary requirements. Table 2 lists data on the nutritional requirements of laboratory animals during growth, gestation, lactation, maintenance, and reproduction as recommended by the National Academy of Sciences (NAS 1974, 1977, 1978). It should be noted that a data gap exists in nutrient requirements for animals involved in long-term studies beyond their reproductive life span (NAS 1978).

Table 1. Approximate Nutrient Composition of EPA, NIH, AIN and Commercial Laboratory Animal Diet Formulations

Nutrients	EPA[a] rodent diet		Agway[b] (Charles River) RMH 3000	Purina[c] Rodent Lab Chow® #5002	Purina[c] Guinea Pig Chow® #5026	NIH open[d] formula mouse & rat diet	Purified diet[e] AIN-76® (rodents)	Purina[c] Canine Diet #5007	Purina[c] Rabbit Chow® #5322
	min.	max.							
Crude protein, min., %	18.0	NG	22.0	20.0	18.0	23.5	17+	25.0	16.0
Crude fat, min., %	4.3	NG	5.0	4.5	4.0	5.0	5	9.0	2.5
Crude fiber,%	4.2	NG	5.0	5.5 (max.)	16.0 (max.)	4.5	5	4.0 (max.)	18.0 (max.)
Ash, %	8.0	NG	6.0	7.0 (max.)	9.0 (max.)	NG	NG	10.0 (max.)	8.0 (max.)
Linoleic acid, %	NG	NG	0.9	NG	NG	NG	NG	NG	NG
Total digestible nutrients (TDN), %	NG	NG	NG	77.0	67.6	NG	NG	78.0	66.0
Amino acids, %									
Arginine, %	0.95	NG	1.63	1.13	1.08	1.25	NG	1.46	0.90
Cystine, %	0.27	NG	0.44	0.27	0.28	0.35	NG	0.37	0.25
Glycine, %	1.00	NG	1.35	0.86	0.87	1.10	NG	1.85	0.77
Histidine, %	0.38	NG	0.51	0.49	0.43	0.50	NG	0.59	0.40
Isoleucine, %	0.95	NG	1.10	1.03	0.96	1.10	NG	1.13	0.82
Leucine, %	1.50	NG	1.83	1.58	1.46	1.80	NG	2.21	1.30
Lysine, %	0.90	NG	1.41	1.18	0.95	1.20	NG	1.10	0.78
Methionine, %	0.38	NG	0.42	0.43	0.40	0.50	NG	0.44	0.35
Phenylalanine, %	0.90	NG	1.13	0.88	0.89	1.10	NG	1.20	0.80
Threonine, %	0.65	NG	0.91	0.78	0.71	0.90	NG	0.90	0.64
Tryptophan, %	0.20	NG	0.27	0.24	0.27	0.25	NG	0.25	0.23
Tyrosine, %	0.60	NG	0.83	0.60	0.59	0.75	NG	0.78	0.75
Valine, %	0.95	NG	1.15	1.05	0.95	1.20	NG	1.24	0.84
Minerals									
Calcium, %	1.15	NG	0.97	0.90	1.1	1.20	0.52	1.8	0.95
Chloride, %	NG	NG	0.15	0.47	0.30	NG	0.156	0.42	0.40
Chromium, ppm	NG	NG	NG	2.09	6.01	NG	2.0	2.34	4.56
Cobalt, ppm	0.80	NG	0.96	0.6	0.38	0.70	NG	0.50	0.38
Copper, ppm	12.0	NG	23.0	13.3	21	15	6.0	13.0	13.0
Fluoride, ppm	NG	NG	35.0	NG	NG	NG	NG	NG	NG
Iodine, ppm	1.85	NG	1.1	1.2	1.6	1.80	0.2	1.70	0.59
Iron, ppm	345.0	NG	289.0	180.0	298.7	250	35.0	241.4	252.6
Magnesium, %	0.20	NG	0.21	0.21	0.35	0.15	0.05	0.24	0.25
Manganese, ppm	140.0	NG	61.7	63.0	121	100	54.0	85.0	44.4
Phosphorus, %	0.90	NG	0.85	0.70	0.6	0.95	0.40	1.0	0.50
Potassium, %	0.80	NG	0.95	0.86	1.2	0.80	0.36	0.71	1.40
Selenium, ppm	0.1	0.6	0.25	NG	NG	NG	0.1	NG	NG
Sodium, %	0.33	NG	0.44	0.30	0.32	0.33	0.102	0.42	0.30
Sulfur, %	NG	NG	NG	NG	NG	NG	333.3	NG	NG
Zinc, ppm	50.0	NG	84.0	52.4	122	45	30.0	48.1	32.5
Vitamins									
Vitamin A, IU/g	15.0	75.00	20.229	17.6	30.0 + 59.9[f]	15.0	4.0	40.0	28 + 46[f]
Vitamin D, IU/g	4.0	10.00	1.045[g]	2.2	3.4	4.0	1.0	4.4	2.3
dl,-Tocopherol (vitamin E), ppm	50.0	NG	55.62	60.0	44.0	35.0	56.6	44.0	44.0
Thiamine,ppm	14.0	NG	11.50	13.3	5.5	14.0	5.34	9.8	3.5
Riboflavin, ppm	7.0	NG	10.38	8.0	6.0	7.0	6.0	4.5	4.5
Niacin, ppm	65.0	NG	72.45	60.0	50.0	80.0	30.0	60.0	33.0
Pantothenic acid, ppm	32.0	NG	21.92	17.0	19.0	20.0	16.0[h]	20.0	19.0
Choline, ppm	1900.00	NG	1542.00	1800.0	1850.0	2000.0	NG	2000.0	1600.00
Pyridoxine, ppm	10.0	NG	8.84	6.0	4.0	10.0	7.0[i]	12.5	4.5
Folic acid, ppm	2.0	NG	1.67	4.0	4.2	4.0	2.0	3.6	1.2
Biotin, ppm	0.3	NG	0.40	0.13	0.30	0.15	0.2	0.18	0.12
Vitamin B_{12}, ppm	0.03	NG	0.06	0.02	0.01	0.03	0.1	0.03	0.01
Vitamin K (Menadione), ppm	3.0	NG	0.97	NG	NG	3.0	0.05	0.57	NG
Inositol, ppm	NG	NG	NG	NG	NG	NG	NG	NG	NG
Vitamin C, ppm	NG	NG	NG	NG	1000.0	NG	NG	NG	NG
For autoclavable diets:									
Vitamin A, IU/g	100.0	NG	28.15	37.0	NG	NG	NG	NG	NG
Thiamin, ppm	70.0	NG	67.32	70.0	NG	NG	NG	NG	NG
Vitamin K (Menadione), ppm	20.0	NG	1.38	8.8	NG	NG	NG	NG	NG

NG = Not given.

[a] Data taken from USEPA (1979). Data on Vit. B_{12} and Vit. A in the autoclavable diet have been converted into ppm and IU/g, respectively.

[b] Data taken from Agway Inc. (n.d.). Rat, mouse, hamster 3000.

[c] Data taken from Ralston Purina Company (1980).

[d] Data taken from NIH (1980).

[e] Data taken from AIN (1977) and NAS (1978).

[f] As carotene.

[g] Vitamin D_3.

[h] Ca salt.

[i] Hydrochloride.

Table 2. Estimated Nutritient Requirements of Laboratory Animals

Nutrient	Rat[a,d]		Mouse[a,e]	Golden	Guinea pig[a,g]		Rabbit[c]	
	Growth, gestation lactation	Maintenance	growth reproduction	Hamster[a,f]	growth	Dog[b,h]	Growth	Maintenance
Protein, %	12.00[i]	4.20[i]	12.5, 18.0	15.0	18.0	20.0	16.0	12.0
Fat, %	5.00	5.00	NG	5.0	<1[g]	4.5	2.0	2.0
Digestible energy, kcal/g	3.8	3.8	NG	4.2	3.0	NG	2.5	2.1
Fiber, %	NG[j]	NG	NG	NG	10.0	NG	10-12	14
Linoleic acid, %	0.6	NG	0.3	NG	NG	0.9	NG	NG
Total digestable nutrients (TDN), %	NG	NG	NG	NG	NG	NG	65	5
L-Amino acids, %								
Arginine	0.60	*l*	0.3	0.76	NG	NG	NG	NG
Asparagine	0.40	*l*	NG	NG	NG	NG	NG	NG
Glutamic acid	4.00	*l*	NG	NG	NG	NG	NG	NG
Histidine	0.30	0.08	0.2	0.40	NG	NG	0.3	NG
Isoleucine	0.50	0.31	0.4	0.89	NG	NG	0.6	NG
Leucine	0.75	0.18	0.7	1.39	NG	NG	1.1	NG
Lysine	0.70	0.11	0.4	1.20	NG	NG	0.65	NG
Methionine	0.60[k]	0.23	0.5	0.32	NG	NG	0.6	NG
Phenylalanine + tyrosine	0.80	0.18	0.4	0.83 + 0.57	NG	NG	1.1	NG
Proline	0.40	*l*	NG	NG	NG	NG	NG	NG
Threonine	0.50	0.18	0.4	0.70	NG	NG	0.6	NG
Tryptophan	0.15	0.05	0.1	0.34	NG	NG	0.2	NG
Nonessential	0.59[m]	0.48[m]	NG	NG	NG	NG	NG	NG
Minerals								
Calcium, %	0.50	NG	0.4	0.59	0.8-1.0	1.0	0.4	*o*
Chloride, %	0.05	NG	NG	NG	NG	NG	0.3	0.3
Cobalt, mg/kg	*l*	*l*	NG	1.1	NG	NG	NG	NG
Magnesium, %	0.04	NG	0.05	0.06	0.1-0.3	0.036	0.03-0.04	0.03-0.04
Phosphorus, %	0.40	NG	0.4	0.30	0.4-0.7	0.8	0.22	*o*
Potassium, %	0.36	NG	0.2	0.61	0.5-1.4	0.5	0.6	0.6
Sodium, %	0.05	NG	NG	0.15	NG	NG	0.2	0.2
Sodium chloride, %	NG	NG	NG	NG	NG	1.0	NG	NG
Sulfur, %	0.03	NG	NG	NG	NG	NG	NG	NG
Chromium, mg/kg	0.30	NG	2.0	NG	0.6	NG	NG	NG
Copper, mg/kg	5.00	NG	4.5	1.6	6.0	6.5	3.0	3.0
Fluoride, mg/kg	1.00	NG	*n*	0.024	NG	NG	NG	NG
Iodine, mg/kg	0.15	NG	0.25	1.6	1.0	1.39	0.2	0.2
Iron, mg/kg	35.00	NG	25.00	140.00	50.0	54.0	*o*	*o*
Manganese, mg/kg	50.00	NG	45.0	3.65	40.0	4.5	8.5	2.5
Selenium, mg/kg	0.10	NG	*o*	0.1	0.1	0.10	NG	NG
Vanadium	NG	NG	*p*	NG	NG	NG	NG	NG
Zinc, mg/kg	12.00	NG	*p*	9.2	20.0	45.0	*o*	*o*

Table 2 (continued)

Nutrient	Rat[a,d] Growth, gestation lactation	Rat[a,d] Maintenance	Mouse[a,e] growth reproduction	Golden Hamster[a,f]	Guinea pig[a,g] growth	Dog[b,h]	Rabbit[c] Growth	Rabbit[c] Maintenance
Vitamins								
A, IU/kg	4,000.00	NG	500.0	3,634.00	20,328.00	4,500.00	580.0	*o*
D, IU/kg	1,000.00	NG	150.0	2,484.00	1,000.00	450.00	*r*	*r*
E, IU/kg	30.00	NG	20.0	3.00	50.0	45.00	40.0	*o*
K_1, μg/kg	50.00	NG	3,000.00	4,000.00	5,000.00	NG	*k*	*k*
Choline, mg/kg	1,000.0	NG	600.0	2,000.0	1,000.0	1,100.0	1,200.0	NG
Folic acid, mg/kg	1.00	NG	0.5	2.00	4.00	0.16	NG	NG
Niacin, mg/kg	20.0	NG	1010	90.0	10.0	10.3	180.0	NG
Pantothenate, mg/kg (Calcium)	8.00	NG	10.0	40.0	20.00	9.0	NG	NG
Riboflavin, mg/kg	3.00	NG	7.0	15.0	3.00	2.0	NG	NG
Thiamin, mg/kg	4.00	NG	5.0	20.0	2.00	0.90	NG	NG
B_6, mg/kg	6.00	NG	1.0	6.0	3.00	0.90	39.0	NG
B_{12}, μg/kg	50.0	NG	0.01	10.0	10.00	20.00	NG	NG
Biotin, mg/kg	*k*	*k*	0.2	0.6	0.3	0.09	NG	NG
Inositol (myo-)	*l*	*l*	*l*	100.0	NG	NG	NG	NG
Vitamin C, mg/kg	*m*	*m*	*m*	*m*	200.00	*m*	*m*	*m*

[a] Data taken from NAS (1978).

[b] Data taken from NAS (1974).

[c] Data taken from NAS (1977)

[d] Based on 90% dry matter.

[e] Unless indicated, requirements listed for growth are the same for reproduction. When indicated, first value listed is for growth, second for reproduction. Values are estimated minimal requirements for conventional mice.

[f] Estimates based on minimal amounts in diets adequate for growth for 6 weeks following weaning.

[g] Minimal requirements.

[h] Nutrient levels selected to meet the requirements of the most demanding life cycle segments, i.e., rapid growth and lactation.

[i] Although not stated by author, presumably none required.

[j] NG = not given.

[k] Not required, produced by bacterial synthesis in intestine.

[l] Not required under conventional laboratory conditions.

[m] Dietary source not required.

[n] Controversial whether or not required.

[o] Required; no quantitative data given.

[p] No data; may be needed in ultraclean environments.

[q] Unsaturated fatty acid.

[r] Probably required; amount unknown.

[s] As ideal protein.

[t] Partially supplied by L-cystine.

[u] Mixture of glycine, L-alanine, and L-serine.

2. NUTRITIONAL REQUIREMENTS FOR LABORATORY ANIMALS - COMPARISON OF THE DATA PROPOSED IN THE EPA GOOD LABORATORY PRACTICE STANDARDS WITH THOSE OF COMMERCIAL PRODUCERS, NIH, AND AIN

Nutritional requirements vary from species to species and also with the physiological state of the animal within the same species. Guinea pigs and rabbits, for instance, can utilize cellulose to some extent (NAS 1977, 1978). This is reflected in the commercial diets for these animals. Purina guinea pig chow and rabbit chow include as much as 16 and 18% crude fiber, respectively (see Table 1). The NAS recommendation for fiber content of the guinea pig and rabbit diets is 10 and 10-12%, respectively (see Table 2). Of the laboratory animals under discussion, guinea pigs are unique in their absolute dependence on a dietary source of vitamin C. All commercial diets for guinea pigs are supplemented with vitamin C (see Table 1). Special and richer diets are often used in commercial reproducing colonies (NAS 1978) because, contrary to general assumption, diets that produce maximum postweaning growth are not necessarily adequate for maximum rates of reproduction.

It is worthwhile to point out that the Appendix B of the USEPA (1979) was probably written with respect to the rat, mouse, and hamster, although these animal species are not specified. It apparently was not written for rodents in general since vitamin C, which is essential in the diet for the guinea pig, is not included in the list of dietary requirements. Also, Purina rodent chow #5002 (Ralston Purina Company 1980) is a misnomer, since this chow, lacking in vitamin C, is not intended for the guinea pig. The proper designation for these chows should mention the names of the animals for which they are intended, as exemplified in NIH (1980) and Agway Inc. (n.d.) (see Table 1).

As Table 1 indicates, commercial feed manufacturers are generally in good agreement with the dietary requirements proposed by EPA for rodent diets (a 10% variance is permitted by EPA). Notable exceptions are manganese (140 ppm vs 61.7 ppm for Agway rat-mouse-hamster 3000 diet) and pantothenic acid (32 ppm vs 17 ppm for Purina rodent chow), vitamin A [100 IU/g (30 ppm) vs 28.15 IU/g (8.44 ppm) for Agway's rat-mouse-hamster autoclavable diet], and vitamin K (20 ppm vs 1.38 ppm for Agway's rat-mouse-hamster autoclavable diet).

Table 2 shows the estimated nutrient requirements for the species of concern in this study as recommended by the National Academy of Sciences (NAS 1974, 1977, 1978). These data support the use of special diets in production colonies. Comparison of the nutrient requirements listed in Table 2 with those proposed by EPA and the dietary composition of commercially available feeds (see Table 1) indicates that for most nutrients, the EPA and commercial levels are generally higher than the estimated requirements recommended by the NAS. This is because the nutrient concentrations in commercial diets and those proposed by EPA are formulated to compensate for losses during the manufacture and storage as well as for differences in biological availability of nutrients.

3. EVALUATION OF DIFFERENT TYPES AND FORMS OF DIETS

The two criteria most widely used for judging the adequacy of a diet are: (1) growth curves and (2) reproduction data (i.e., litter size, average weight at birth, average number weaned, average weaning weight, and estrus cycle). Normal growth data for hamsters, rats, and mice are presented in Table 3, and growth curves for rabbits, guinea pigs, and dogs are shown in Figures 1, 2, and 3, respectively. Newberne (1982) has suggested that the study of the subchronic effect of a compound for 90 days should be used to establish dose levels that will result in no more than 10% depression in body weight gain. Reproduction and life span data for these animal species are shown in Table 4. Various types of laboratory animal diets are described in the following sections. A carefully designed long-term toxicity experiment with laboratory animals should take into account the possibility of induction of enzymes by the dietary components and/or contaminants. Such enzymes may have the potential to alter the toxicity of the chemical under investigation (Newberne 1975). This topic is discussed in Section 9.4.

3.1 Types of Diets - Advantages and Disadvantages

3.1.1 Natural-Ingredient Diets

These diets are economical and are the most widely used. They are made from appropriately processed whole grains such as wheat, corn, oats, or commodities that have been subjected to limited amounts of refinement such as fish meal, soybean meal, or wheat bran. Some of the disadvantages are: the necessity for commercial manufacture; the inability to completely control the nutrient concentrations; batch-to-batch variation in nutrient concentration; difficulty in altering composition to study the effect of the absence of a particular nutrient; and the potential for contamination with pesticide residues, heavy metals, or other agents that might alter the response to experimental treatment (NAS 1978).

3.1.2 Purified Diets

Diets formulated with refined ingredients such as casein, sugar, starch, vegetable oil, and cellulose are known as purified diets. Some of the advantages are that (1) ease of preparation in the laboratory, (2) capability for duplication of nutrient concentrations or alteration for induction of nutritional deficiencies or excesses, and (3) low potential for chemical contamination. Disadvantages include being more expensive than natural-ingredient diets and not palatable to all species of animals (NAS 1978). Purified diets can be both open formula and closed formula diets.

3.1.3 Chemically Defined Diets

These diets are prepared by mixing chemically pure compounds such as amino acids, sugars, triglycerides, essential fatty acids, inorganic salts, and vitamins. Two advantages are ease of preparation in the

Table 3. Normal Growth Rate of the Hamster, Rat, and Mouse

Animal species	Range and average body weights (g) as a function of age (days)													
	21		28		42		58		84		112		168	
	range	av.	range	av.	range	av.	range	av.	range	av.	range	av.	range	av.
Outbred Golden Hamsters														
Cr:RHG (SYR)														
Male	29-51	40	32-71	49	NG[c]	NG	86-98	92	99-109	104	NG	NG	128-142	141
Female	30-50	40	31-69	44	NG	NG	85-104	95	103-127	115	NG	NG	150-167	158
Rats: Sprague-Dawley														
Male	50-61	46[a]	71-114	75[a]	NG	NG	206-259	236[a]	317-385	302[a]	NG	NG	436-482	365[a]
Female	45-63	44[a]	71-130	64[a]	NG	NG	188-205	185[a]	231-283	210[a]	NG	NG	275-301	230[a]
Rats: Fischer														
Male	24-39	NG	28-56	53[a]	NG	NG	155-209	160[a]	183-238	213[a]	NG	NG	312-380	256[a]
Female	22-39	NG	35-51	44[a]	NG	NG	105-156	123[a]	112-175	145[a]	NG	NG	203-238	162[a]
Inbred mouse:														
Male	7.7-15.8[b]	11.0	11.3-20.5[b]	15.7	14.4-28.7[b]	21.0	16.1-30.8[b]	24.1	NG	NG	23.2-35.7[b]	30.2	NG	NG
Female	7.5-14.8[b]	10.2	9.9-18.6[b]	14.2	13.1-25.8[b]	18.6	15.1-28.9[b]	21.5	NG	NG	21.1-37.6[b]	28.1	NG	NG

[a] Average body weights are averages of figures given by two different suppliers of the two strains.
[b] Range of averages for 26 inbred mouse strains.
[c] Not given.

Source: NAS (1978).

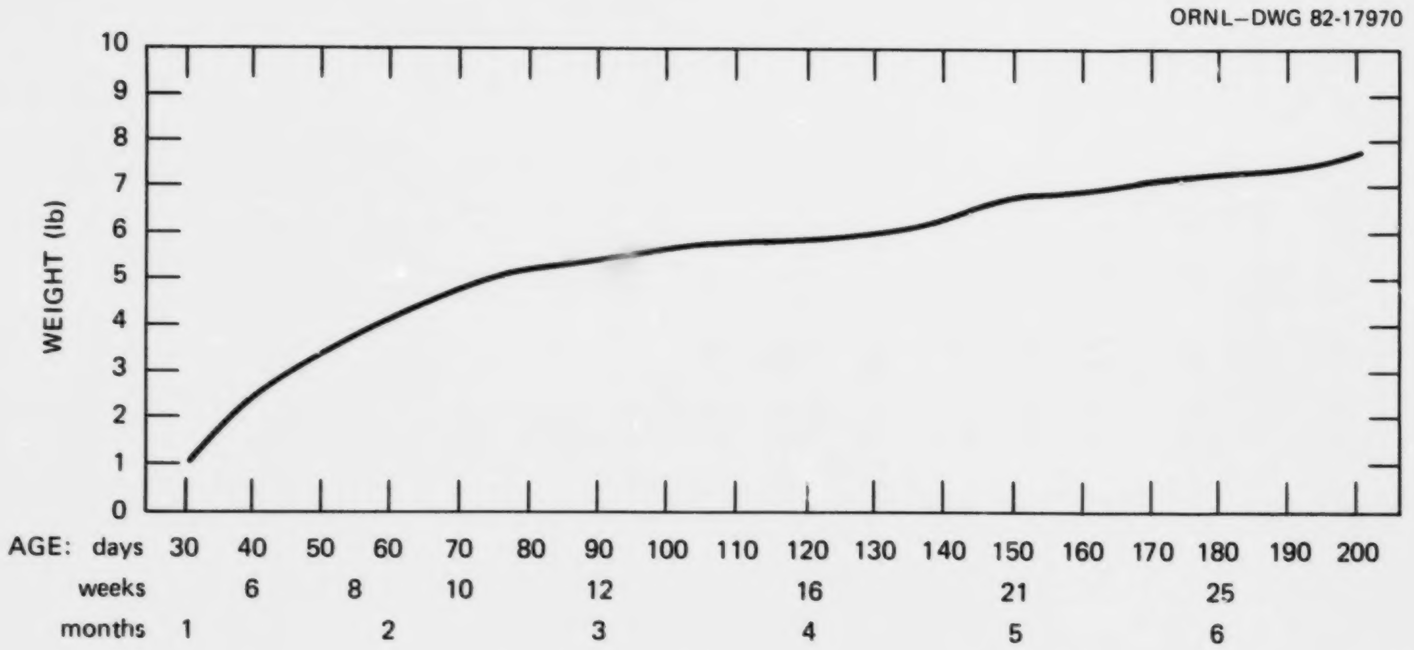


Figure 1. Growth curve for rabbit (New Zealand White). (Source: Dutchland Laboratories, Inc. 1982.)

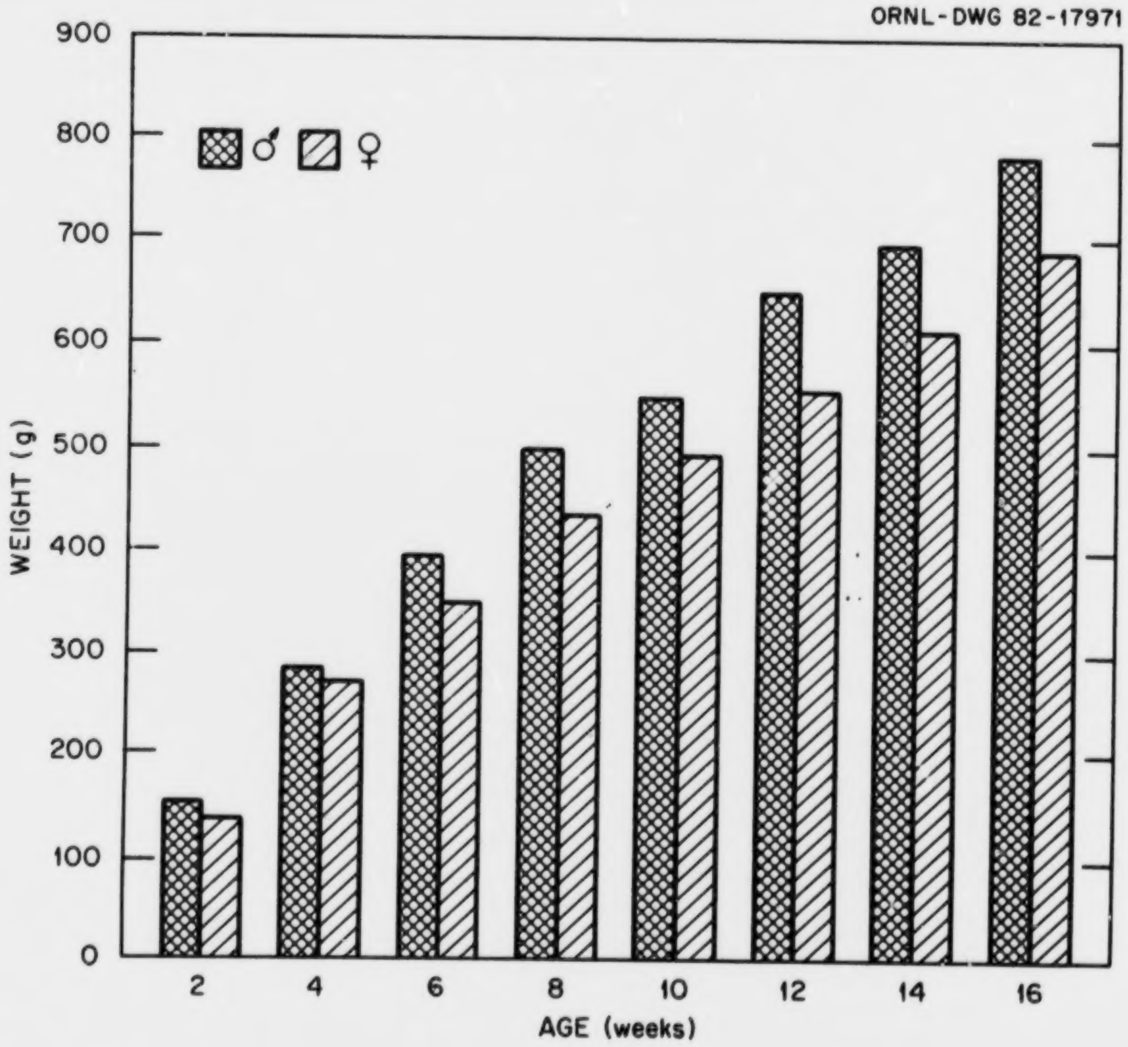


Figure 2. Growth data for guinea pig (Ft. Detrick Dunkin Hartley). (Source: Dutchland Laboratories, Inc. 1982.)

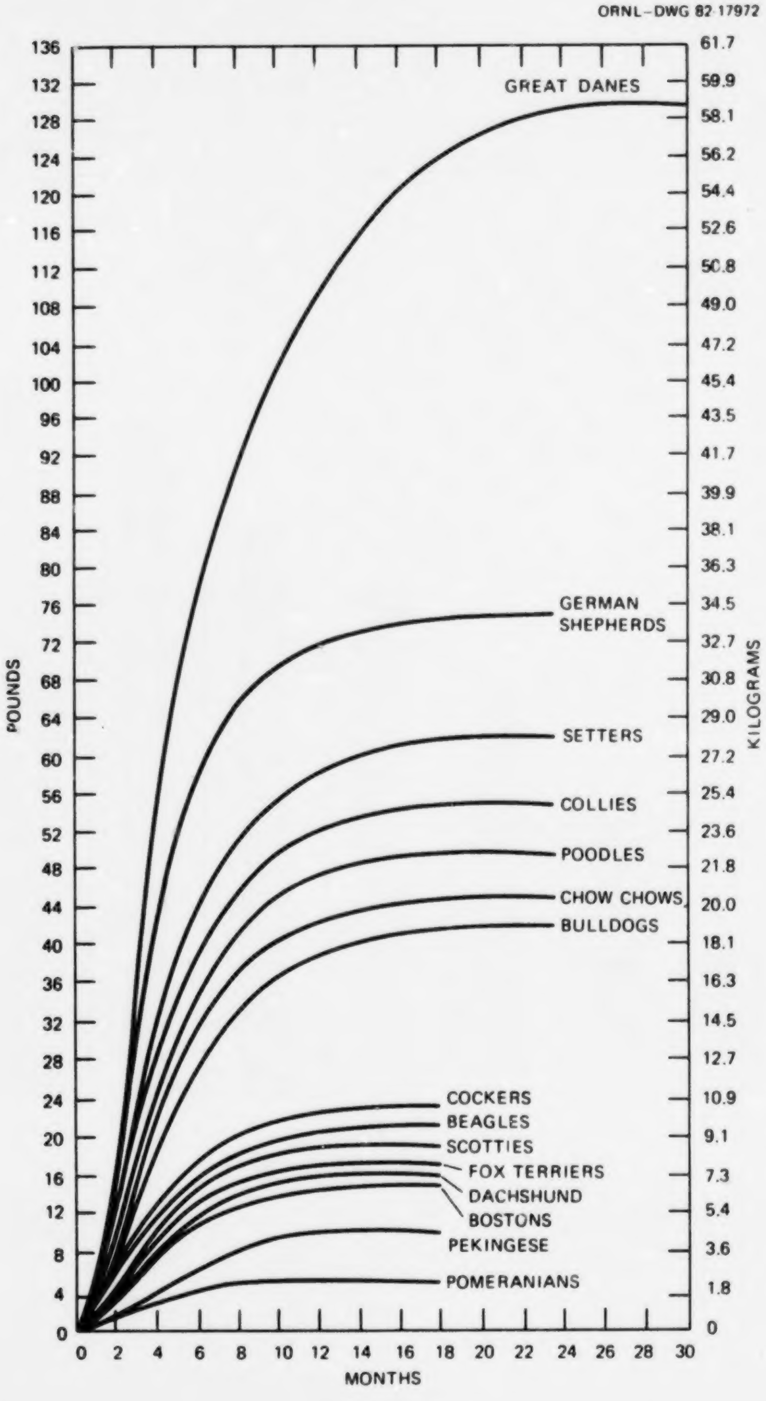


Figure 3. Growth curves for fifteen breeds of dogs. (Source: NAS 1974, p. 1.)

Table 4. Reproduction[a] and Life Span[b] Data on Selected Laboratory Animals

	Hamsters	Mice	Rats	Dogs	Guinea pigs	Rabbits
Weight						
Adult male	85-110 g	20-40 g	300-400 g	13.-18.5 kg (beagle)	1000-2000 g	4.5-5 kg
Adult female	95-120 g	25-40 g	250-300 g	13.5-16 kg	850-900 g	4.5-6.5 kg
At birth	2 g	1-5 g	5-6 g	350-450 g	100 g	100 g
Breeding Age						
Male	60 days/85-110 g	50 days/20-35 g	100 days/300 g	10-12 months	90-150 days/550 g	6-7 months/4 kg
Female	60 days/95-120 g	50-60 days/20-30g	100 days or 200 g	9-12 months	90-150 days/500 g	5-6 months/4.5 kg
Breeding Data						
Estrus cycle	4-15 days	4-5 days	5 days	bi-annually	post partum 16-18 days	polyestrus
Gestation	15-19 days	17-21 days	20-22 days	60-65 days	59-67 days	30-32 days
Weaning age	20-24 days/35 g	16-21 days/10-12 g	21 days/40-50 g	6-8 weeks	10 days/250 g	8 weeks/1.8 kg
Litter size	6-10	1-23/av. 12	8-12	4-8	1.6/av. 4	1-18/av. 8
Breeding life						
Male	1 year	18 months	1 year	6-14 years	5 years	1-3 years
Female	10-12 months/4-5 litters	6-10 litters	1 year	6-10 years	4-5 years	1-3 years
Rebreeding	4-6 days	immediately	immediately	next heat period	immediately	35 days
Mating	Pair	Pair or colony; 1 male, 3 females	Pair or colony 1 male, 3-4 females	1 male, up to 40-60 females	Colony: 1 male, 3-10 females	Pair. Mate in bucks cage; 1 male, 6-10 females
Life span	1 yr. av., 2 yr. max.[b]	1.5 yr. av., 3 yr. max.[b]	3 yr. av., 4 yr. max.[b]	15 yr. av., 35 yr. max.[b]	3 yr. av., 7 yr. max.[b]	6 yr. av. 15 yr. max.[b]

[a] Data taken from Ralston Purina Company (n.d.).

[b] Data taken from Agway Inc. (n.d.).

laboratory and strict control of nutrient concentrations. The fact that these diets are too expensive for general use and that availability of nutrients may be altered by oxidation or interactions among nutrients represents the principal disadvantages (NAS 1978).

3.1.4 Open Formula Diets

These are diets of known ingredient composition and are manufactured from natural, purified, or chemically defined ingredients. Specifications for open formula diets not only state the amount of each ingredient to be used but also define the minimum acceptable nutrient composition of ingredients to be used. Advantages include the fact that the diet can be procured by competitive bidding, resulting in lower cost compared with the brand name diets under sole source negotiated contracts, and that the investigator has the option to make alterations in nutrient composition to satisfy specific program objectives (NIH 1980).

3.1.5 Closed Formula Diets

Most widely used closed formula diets are natural-ingredient diets, but the ingredient compositions are well-kept secrets of the manufacturers. In general, most laboratory animals thrive on these diets. Some disadvantages are that these diets introduce an unknown element (such as nature and composition of ingredients) that can be critical to the interpretation of the results in a particular investigation and that the diets can be procured only through negotiated sole source contracts. A list of ingredients as well as the nutrient composition of the diet is provided by the manufacturer (NIH 1980). These diets have extensive historical background data. Recently, Newberne (1982) reported wide variation in the nutrient composition of closed formula diets, and toxic component composition of natural ingredient diets from commercial sources has been reported (Table 5).

3.1.6 Quasi-Open Formula Diets

The closed formula diet can be procured through competitive bidding by specifying a list of acceptable ingredients and thereby constitutes a quasi-open formula diet. However, the manufacturer is not obligated to use all of the listed ingredients (NIH 1980).

3.2 Forms of Diets

Diets in various physical forms have been used for laboratory animals. These include (a) meal, (b) pelleted, (c) crumbled, (d) extruded, (e) baked, (f) flaked, (g) semimoist or gel forms, and (h) liquid. For a detailed discussion of these different forms of diet, the reader is referred to the document published by the National Academy of Sciences (NAS 1978). The pelleted, extruded, gel, and liquid forms of diets are discussed below.

The pelleted diet is the one most widely used for feeding laboratory animals. During the pelletization process, meal is heated to 180-190°F for 30 seconds to form dense compressed pellets that are easier to

Table 5. Concentrations of selected nutrient and toxic components in random samples of rodent natural product diets

Component	Requirements, rat[a]	Diet analysis[b]
Minerals		
Calcium (%)	0.56	0.5, 0.27, 1.67
Phosphorus (%)	0.44	1.90, 0.68, 0.13
Magnesium (%)	0.04	0.54, 0.02, 0.23
Copper (ppm)	5.60	5.8, 6.9, 65
Zinc (pppm)	13.3	20, 17, 10, 60
Amino acids		
Tryptophan (%)	0.17	0.10, 0.39, 0.08
Methionine (%)	0.67	0.13, 1.21, 0.22
Phenylalanine + tyrosine (%)	0.89	2.01, 1.05, 1.82
Vitamins		
Vitamin A (mg/kg)	0.67	2.10, 0.31, 3.75
Vitamin D (IU/kg)	1108	987, 1360, 5100
Riboflavin (mg/kg)	0.5-4.0	12, 0.3, 0
Toxicants		
Aflatoxins (ppm)	—	0.01, 0.20, 0.12, 0.0
Nitrates (ppm)	—	90, 5, 10, 18, 32
Nitrosamines (ppb)		
N-dimethylnitrosamine	—	8, 0, 32, 18
N-nitrosopyrrolidine	—	0, 7, 5, 16
Lead (ppm)	—	0.8, 1.8, 4.2, 2.5

[a] Requirements according to the National Research Council, 1978*a*.

[b] Each line represents different samples from the same manufacturer. Analysis conducted at MIT over a period of 15 years.

Source: Newberne 1982.

handle and store. Wastage is minimized, and the daily dietary intake of the test animals is relatively easy to measure. However, if administered in the diet, the substance under study has to be incorporated in the meal before the pellets are made.

During the manufacture of the extruded diet, the meal is subjected to moist heat (350-400°F for 1 minute). This treatment partially cooks the starch and makes the diet highly palatable to some animals, e.g., nonhuman primates, dogs, cats. However, other laboratory animal species waste a lot of feed, and frequent feeding is necessary because of the low diet density.

Diets in semimoist or gel forms are produced by adding water, agar, gelatin, or other jelling agents, to meal. These diets are more palatable than dry rations and more convenient for incorporating the substances to be tested. They also allow efficient measurement of food consumption. However, because these diets are susceptible to microbial growth, they must be kept frozen or refrigerated and feeding must be at frequent intervals. Large quantities are thus difficult to handle.

Following the pioneering liquid diet research of Lieber and DeCarli (1966), Bio Serve Inc. has been offering isocaloric liquid diet formulations for rats, mice, and dogs. This diet offers an excellent delivery option in toxicity studies of volatile test substances.

3.3 Autoclaving of Diets

Extent of loss of heat sensitive components of the diet, e.g., vitamins, is partly determined by the procedure used during autoclaving. It is perhaps desirable to recommend a standardized procedure in the EPA Good Laboratory Practice (USEPA 1979) for autoclaving laboratory animal feed. One procedure, used by Agway (Agway Inc. n.d.), is outlined below.

> **PROCEDURES FOR STERILIZATION OR PASTEURIZATION OF R-M-H 3500**
>
> Since autoclaves differ, these procedures will be outlined in three (3) generalized phases so that the operator of any particular autoclave may successfully sterilize or pasteurize the formula based on operational procedures peculiar to that autoclave.
>
> **PHASE I. CREATE VACUUM AND DEVELOP HEAT**
>
> Adjust jacket pressure to normal. Place volume of pelleted feed in autoclave according to its capacity, then pull a vacuum up to 25 to 29 inches for approximately 15 minutes. Afterward, slowly meter in steam while maintaining a vacuum of 10 to 15 inches. The interval of time required should be sufficient to bring the pellets to a heat of 180 to 190°F (10 to 15 minutes). Meter steam in slowly so that the pellets are brought to the above temperature and yet moisture is not

permitted to condense on or within the pellets. Experimentation may be required.

PHASE II. PASTEURIZATION

Turn off vacuum pump. Introduce full volume of steam. This should elevate the temperature within the pellets to 215 to 220°F. This will require approximately 20 to 25 minutes from the time steam pressure reaches 15 lbs.

PHASE II. STERILIZATION

Turn off vacuum pump. Introduce full volume of steam. This should elevate the temperature within the pellets to 250 to 260°F. This will require approximately 20 to 25 minutes from the time steam pressure reaches 15 lbs.

PHASE III. EXHAUST AND DRY

Open chamber exhaust and vent to 2 to 5 lb. of steam pressure. Turn on vacuum pump pulling 20 to 25 inches to remove steam from the autoclave. The drying cycle (approximately 30 minutes depending on capacity of vacuum pump and quantity of feed) may be adjusted according to desired dryness. Stop vacuum pump and introduce bacteriologically filtered air into the chamber.

3.4 Discussion

Highly purified diets and chemically defined synthetic diets are the diets of choice when true and reliable standard diets are required over an indefinite period. However, these diets have two main disadvantages — they are expensive and they are not consumed well by all species. The use of the natural-ingredient diets of known composition (open formula) has been advocated (AIN 1977). Admittedly there is seasonal and year-to-year variation in the composition of the natural ingredients but by specifying the standards of the quality of the ingredients, the variability can be kept to a minimum. The list of approved ingredients should be revised from time to time to allow inclusion of new, improved, or more economical substituents. However, as Knapka (1974) has pointed out, "the use of open formula rations could result in a liability without adequate control of product quality and sanitation of manufacturing facilities. Quality assurance at NIH is obtained by requiring that the facilities used to manufacture the ration be in compliance with the sanitation standards in the National Institutes of Health Standard Number 1" (NIH 1980). It is imperative that other animal feeds containing drugs (e.g., cattle feed, poultry feed) should not be manufactured on the same premises where laboratory animal feed is manufactured to avoid possible cross contamination.

The closed formula diet on the other hand has a history of acceptable performance although the investigator is left in the dark so far as the composition of the natural ingredients is concerned. These diets are also generally more expensive than the open formula diets. The manufacturers closely guard the secrecy of the diet formula, and they are free to alter the composition of the ingredients according to their availability and prices (AIN 1977). It will be very difficult to unearth any interaction between dietary components and the test substance in experiments using closed formula diets. Although the open formula diet need further development and improvement, compared with the closed formula diet, the ability to control the diet composition will certainly aid in obtaining sound and reproducible animal experiments. The National Institutes of Health are currently using open formula diets for the rat, mouse, hamster, guinea pig, and rabbit. The food for dogs, however, is purchased via an advertised contract using a specification for a quasi-open formula diet. Manufacturers may use any combination of the listed ingredients to produce a diet with the indicated nutrient concentrations (NIH 1980).

Recent data indicate that highly purified diets are not infallible, although the use of such diets for definitive studies has been strongly recommended (Newberne 1982). The AIN-76A^{TM} diet, for example, has been reported to cause severe periportal lipidosis in the livers of male F-344 or CD rats (Hamm et al. 1982). The AIN-76^{TM} diet causes nephrocalcinosis in female Sprague-Dawley rats (Nguyen 1982). The presence of traces of solvents used in the purification of some of the ingredients in semisynthetic diets may also cause complications.

Assuming that a perfect diet, free from contaminants, preservatives, etc., is used in long-term toxicity testing with animals, one cannot help but raise the question of how relevant will data from such tests be for assessing the risk to man, who is normally fed his share of preservatives, pesticide residues, etc. It appears that the practical solution to this problem is to establish adequate quality control over nutrients and contaminants and rigid protocols in natural ingredient diets to ensure reproducibility of results free from any interference due to dietary factors, no matter whether closed formula or open formula diets are used in the experiment. Newberne and McConnell (1981) have also strongly recommended surveillance over both contaminants and nutrients in natural product as well as semipurified diets. This has become extremely important in view of the fact that closed formula diets have been found to differ considerably in the composition of nutrients and contaminants (Newberne 1982, Table 5).

4. DIETARY INTAKE

During the long-term toxicity test, it is not unusual for an animal to lose or gain weight at a lower than normal rate. The weight loss or reduced rate of weight gain may be due to low dietary intake or to the toxic effect of the chemical or to both. Excessive weight in laboratory animals should also be avoided since the response to chemical stress of an overweight animal is likely to be different from that of a normal animal. It is critical, therefore, to monitor the food intake of the test animals. Equal food intake between control and test animals can be achieved by pair feeding (Newberne et al. 1978). Most laboratory animals are coprophagous, which introduces an unknown element in the diet through ingestion of soiled bedding. This factor however, can be minimized by the use of screen-bottomed rather than solid-bottomed cages (Newberne 1978).

The effects of dietary restrictions on immunity and aging have been reported by Weindruch and Makinodan (1981). Undernutrition without malnutrition has led to increased life-span and lower spontaneous cancer incidence in mice (Weindruch and Walford 1982). What is true for mice may be true for humans, but it may not be wise to do long-term toxicity studies with laboratory animals on restricted diets since this will introduce an additional factor in extrapolating the results of animal experiments to human exposure. Besides, administration of restricted diets to a large number of laboratory animals is expensive and difficult.

5. VITAMINS AND MINERALS USED IN ANIMAL DIET FORMULATIONS

5.1 Vitamins

The guinea pig is unique among the laboratory animal species under discussion (mouse, rat, hamster, guinea pig, rabbit, and dog) in its dietary requirement for vitamin C (NAS 1978). Most other animals can synthesize sufficient amounts of vitamin C to meet their daily requirement so the vitamin does not need to be added to their diet. Unfortunately, the ascorbic acid in the pelleted feed is not stable. This phenomenon has been investigated at Hoffmann-La Roche (n.d.) using crystalline ascorbic acid and ethylcellulose-coated ascorbic acid. Fifty-one percent of the ethylcellulose-coated ascorbic acid is retained after storage for 3 months at room temperature whereas only 27% of the uncoated crystalline ascorbic acid was retained under the same conditions. Ethylcellulose-coated ascorbic acid is used in NIH open formula diets (NIH 1980). Additional ascorbic acid supplementation of the feed is necessary if the feed is to be used during the second 3-month period of storage.

The preservative ethoxyquin is used as a stabilizer for vitamin A in both the NIH open formula diet and commercial diets (see Sect. 7.4). Preservatives and their use for this purpose should be considered for inclusion in the proposed EPA guidelines. Present EPA guidelines (USEPA 1979) do not permit the use of any preservative in the animal feed.

Inositol is not normally required in the diet of laboratory animals; however, the National Academy of Sciences recommends a level of 100 ppm in the diet of the golden hamster (NAS 1978). Inositol may also be required under special circumstances, e.g., rodents kept on antibiotics (NAS 1978).

Table 6 lists sources of vitamins included in the EPA GLP (USEPA 1979), NIH open formula rations for mouse/rat and germ-free mouse/rat (NIH 1980), AIN-76™ purified mouse and rat diet (AIN 1977), Purina Rodent Chow #5002 and autoclavable Rodent Chow #5014 (Ralston Purina Company 1980), and Agway R-M-H 3000 and autoclavable R-M-H 3500 (Agway Inc. n.d.). Choline bitartrate and thiamine hydrochloride have been used in the AIN-76™ purified diet in place of choline chloride and thiamine mononitrate, respectively. The latter two sources are included in the EPA GLP (USEPA 1979) and are also constituents of commercial diets. Consideration should be given to the inclusion of choline bitartrate and thiamine hydrochloride in the proposed guidelines (USEPA 1979). Purina Rodent Chows #5002 and #5014 apparently do not include any source of biotin and probably depend on the natural occurrence of biotin in feed ingredients.

5.2 Minerals

Table 6 lists sources of minerals included in the EPA GLP (USEPA 1979), NIH open formula rations for mouse/rat and germ-free mouse/rat (NIH 1980), AIN-76™ purified mouse and rat diet (AIN 1977), Purina

Table 6. Vitamins, Minerals, and Animal Diet Formulations[a]

		NIH[c]			Purina[e]		Agway[f]	
	EPA GLP[b]	Mouse/rat diet	Germ-free mouse/rat diet	AIN-76® purified diet[d]	Rodent chow #5002	Autoclavable chow #5014	R-M-H 3000	R-M-H 3500 (autoclavable)
A. Vitamins								
Vitamin A acetate	✓	✓	✓	✓			✓	✓
Vitamin A palmitate	✓	✓	✓	✓				
Vitamin D activated animal sterol (D_3	✓	✓	✓	✓	✓	✓	✓	✓
Menadione activity	✓	✓	✓	✓				
dl,α-Tocopherol acetate	✓	✓	✓	✓				
Choline bitartrate				✓				
Choline chloride	✓	✓	✓		✓	✓	✓	✓
Folic acid	✓	✓	✓	✓	✓	✓	✓	✓
Niacin	✓	✓	✓	✓	✓	✓	✓	✓
d-Calcium pantothenate	✓	✓	✓	✓	✓	✓	✓	✓
Riboflavin supplement	✓	✓	✓	✓	✓	✓	✓	✓
Thiamine mononitrate	✓	✓	✓		?[g]	?[g]	✓	✓
B_{12} supplement	✓	✓	✓		✓	✓	✓	✓
Pyridoxine hydrochloride	✓	✓	✓	✓	✓	✓	✓	✓
d-Biotin	✓	✓	✓	✓			✓	✓
Inositol								
Vitamin C								
Menadione sodium bisulfite						✓	✓	✓
Cyanocobalamine (Vit. B_{12})				✓				
Vitamin A supplement					✓	✓		
Thiamine hydrochloride				✓	?[g]	?[g]		
Vitamin E supplement					✓	✓	✓	✓
B. Minerals								
Cobalt carbonate	✓	✓	✓		✓	✓	✓	✓
Copper sulfate	✓	✓	✓		✓	✓		
Copper oxide							✓	✓
Cupric carbonate				✓				
Iron sulfate	✓	✓	✓				✓	✓
Ferrous carbonate					✓	✓	✓	✓
Ferric citrate				✓				
Magnesium oxide	✓	✓	✓	✓		✓	✓	
Zinc oxide	✓		✓		✓	✓	✓	✓
Zinc sulfate					✓	✓		
Zinc carbonate				✓				
Calcium iodate	✓		✓		✓	✓	✓	✓
Calcium carbonate (ground limestone)	✓	✓	✓		✓	✓		
Monocalcium phosphate				✓				
Dicalcium phosphate	✓	✓	✓		✓	✓	✓	✓
Salt	✓	✓	✓	✓	✓	✓	✓	✓
Silicon dioxide						✓		✓
Manganous oxide	✓		✓		✓	✓	✓	✓
Manganous carbonate				✓				
Potassium iodate				✓				
Potassium citrate				✓				
Potassium sulfate				✓				
Sodium selenite				✓				
Chromium potassium sulfate				✓				

[a] ✓ indicates the use in animal diet formulation.

[b] USEPA (1979).

[c] NIH (1980).

[d] AIN (1977).

[e] Ralston Purina Company (1980).

[f] Agway (n.d.), Rat-Mouse-Hamster diet.

[g] Source of thiamine not specified.

Rodent Chow #5002 and autoclavable Rodent Chow #5014 (Ralston Purina Company 1980), and Agway R-M-H 3000 and autoclavable R-M-H 3500 diets (Agway Inc. n.d.). As Table 6 indicates, the sources of these minerals other than those specified in the USEPA (1979) include the following: copper oxide, cupric carbonate, ferrous carbonate, ferric citrate, zinc sulfate, zinc carbonate, monocalcium phosphate, manganous carbonate, potassium iodate, citrate, sulfate, sodium selenite, and chromium potassium sulfate. In addition to the latter five sources mentioned above, monocalcium phosphate, zinc carbonate, ferric citrate, and cupric carbonate are used only in the AIN-76™ purified mouse amd rat diet. A purified diet requires supplementation with trace elements because essentially none are provided in the major ingredients. Although chromium is required in the purified AIN-76™ diet and in the NAS recommended guinea pig diet (Tables 1 and 2 respectively), it is not used in Ralston Purina or Agway Inc. commercial diet formulations (Table 1). Silica is used as an anticaking agent in autoclavable diets - Purina Rodent Chow #5014 and Agway R-M-H 3500. Most of the diet formulations except the purified diet depend on the natural presence of selenium in the feed ingredients and do not include any extraneous selenium in the form of sodium selenite, although the addition of extraneous selenium has been permitted by the EPA as an exception to the Delaney clause (see Sect. 10.3). Copper oxide, cupric carbonate, ferrous carbonate, iron ammonium citrate, zinc sulfate, zinc carbonate, manganese carbonate, and potassium iodate are included in the GRAS list (USFDA 1980a) (Table 7). Consideration should be given to expansion of the list of mineral sources in USEPA (1979) to include these different mineral forms and silica, allowing some flexibility in the diet formulation.

Table 7. Trace minerals included in the GRAS List[a]

Element	Source compounds
Cobalt	Cobalt acetate Cobalt carbonate Cobalt chloride Cobalt oxide Cobalt sulfate
Copper	Copper carbonate Copper chloride Copper gluconate Copper hydroxide Copper orthophosphate Copper oxide Copper pyrophosphate Copper sulfate
Iodine	Calcium iodate Calcium iodobehenate Cuprous iodide 3,5-Diiodosalicylic acid Ethylenediamine dihydroiodide Potassium iodate Potassium iodide Sodium iodate Sodium iodide Thymol iodide
Iron	Iron ammonium citrate Iron carbonate Iron chloride Iron gluconate Iron oxide Iron phosphate Iron pyrophosphate Iron sulate Reduced iron
Manganese	Manganese acetate Manganese carbonate Manganese citrate (soluble) Manganese chloride Manganese gluconate Manganese orthophosphate Manganese phosphate (dibasic) Manganese sulfate Manganous oxide
Zinc	Zinc acetate Zinc carbonate Zinc chloride Zinc oxide Zinc sulfate

[a] These substances added to animal feeds as nutritional dietary supplements are generally recognized as safe when added at levels consistent with good feeding practice.

Source: USFDA (1980a).

6. INGREDIENTS USED IN LABORATORY ANIMAL DIETS

All of the ingredients listed in Appendix B of the EPA Good Laboratory Practice Standards, July 26, 1979 (USEPA 1979), are used in the NIH and commercial diet formulations (see Table 8). However, some ingredients are not included in the above-mentioned list but are used in both NIH and commercial diet formulations. These include animal fat protected with butylated hydroxyanisole (BHA), cane molasses, meat and bone meal, and dried skimmed milk. In addition, the commercial diets include casein, dried whey, ground beet pulp, and wheat germ meal, which are not used in the NIH diet (Table 8). The NIH dog diet formulations allow the use of the following preservatives in various ingredients: BHA, propylene glycol, propyl gallate, and citric acid (NIH 1980). Animal feeds containing animal fat without preservative have a shelf life of about 12 weeks when stored at room temperature (personal communication from Dr. John C. Chah, Ziegler Bros. 1982). However, when soy oil is used as a source of fats, it contains enough natural antioxidants (tocopherols) to obviate the necessity of adding any extraneous preservative.

From the available information it seems that none of the feed manufacturers have any control over the supply of the food ingredients, although this is where the most effective control on the major source of contaminants in the feed can be exercised. It would seem advisable to include in a given set of guidelines the specifications (grade, etc.) for individual ingredients. To illustrate the point consider the NAS specification for wheat (NAS 1974). As many as six different types of wheat have been listed as common dog food ingredients: (1) wheat, durum, *triticum durum*, (2) wheat, hard red spring, *triticum aestivum*, (3) wheat, hard red winter, *triticum aestivum*, (4) wheat red spring, *triticum aestivum*, (5) wheat, soft, *triticum aestivum*, and (6) wheat, soft red winter, *triticum aestivum*. These vary considerably in their nutritional quality (e.g., protein content varies from 12 to 16.3% on dry basis). The NIH open formula diet avoids all these uncertainties by specifying hard winter wheat (NIH 1980).

The other source of contaminant control is the guidelines for proper sanitation in facilities used to manufacture the ration (NIH 1980, Appendix A, Standard 1).

Table 8. Ingredients Commonly Used in Laboratory Animal Diets

Ingredient	Agway R-M-H 3000	Purina 5002 certified rodent chow	Purina canine diet #5007	Purina guinea pig chow #5026	NIH open formula mouse & rat diet
A. Ingredients listed in the EPA GLP (USEPA 1979)					
Alfalfa meal	✓	✓		✓	✓ (17% protein dehydrated)
Brewers dried yeast	✓	✓	✓		✓
Corn gluten meal			✓		✓ (60% protein)
Dicalcium phosphate	✓	✓	✓	✓	✓
Fish meal	✓	✓	✓		✓
Ground whole wheat	✓	✓	✓	✓	✓ (hard winter wheat)
Ground #2 yellow shelled corn	✓	✓Ground extruded corn	✓Ground yellow corn	✓Ground yellow corn	✓
Ground whole oats		✓Ground oat groats		✓	
Ground limestone		✓	✓	✓	✓
Salt	✓	✓	✓	✓	✓
Soy oil		✓			✓
Soybean meal	✓	✓	✓	✓	✓ (49% protein)
Wheat middlings	✓	✓	✓	✓	✓
B. Ingredients not listed in the EPA GLP (USEPA 1979) but used in commercial diets					
Ground beet pulp		✓	✓		
Wheat germ meal		✓	✓		
Cane molasses		✓		✓	✓dry molasses
Dried whey	✓	✓	✓dried milk products	✓	
Dried skimmed milk	✓				✓
Casein		✓			
Animal fat & BHA	✓		✓	✓	✓Used in dog food
Meat & bone meal	✓		✓		✓Used in dog food

✓ Indicates inclusion in the particular diet formulation.

7. CONTAMINANTS AND PRESERVATIVES IN ANIMAL FEEDS

7.1 Introduction

Contaminants in laboratory animal feeds arise from biological as well as nonbiological sources. Biological contaminants originate from microorganisms growing on plants in the field, grain, and prepared feed. These are mainly estrogens, aflatoxins, and trichothecene toxins. Nonbiological contaminants are usually pesticide residues and nitrosamines.

When laboratory animals consume any contaminant residue daily in their diet, they are in effect chronically exposed to a considerable amount of the contaminant over their lifetime; for instance, when a rat is exposed to 100 ppb of lindane in its daily dietary intake of 12 g, the total chronic exposure over its life-span of 3 years amounts to about 1.3 mg. These dietary contaminants have the potential for affecting the outcome of long-term animal experiments in various ways. They may act as carcinogens, cocarcinogens, cancer promotors, teratogens, mutagens, or general toxicants. They may also induce the production of microsomal enzymes that may affect the metabolic pattern and inhibit neoplasia (see Sect. 9.4). In either case, both inhibitors and enhancers of toxicity are present in the natural ingredient diet. These diets thus differ from semipurified diets in the way they 'permit' an animal to respond to a test substance, usually in the animal's favor (Newberne and McConnell 1981). This protective action may have a good or bad effect on experimental results, depending on the purpose of the study. This effect extends to spontaneous as well as chemically induced tumors. If the objective is to accumulate animal data for extrapolation to assess the toxicity to man, the presence of these dietary contaminants in the animal feed may not be bad since it is practically impossible for humans to consume food completely free from pesticide residues.

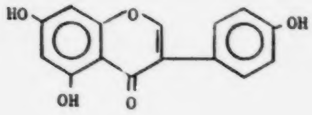
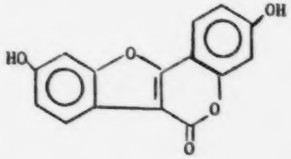
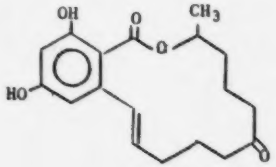
Preservatives are chemicals deliberately added to animal feed to increase its shelf life and preserve the easily oxidizable vitamins present in the feed. The following subsections will describe in more detail biological contaminants, nitrosamines, and preservatives and will examine the differences between EPA's proposed contaminant tolerances for rodent diets and the contaminant levels found in commercial rodent feeds.

7.2 Biological Contaminants

7.2.1 Estrogens

Table 9 lists three estrogens that may occur in animal feed. These are formed when a crop is infected with fungi in the field or during storage. The three estrogens in Table 9 show a low level of estrogenic activity in the following order: genistein > coumesterol > zearalenone. Therefore, they are usually not of much concern in animal feed manufacture. However, when animals are used for studies on reproduction, the estrogenic activity of the feed should be assayed.

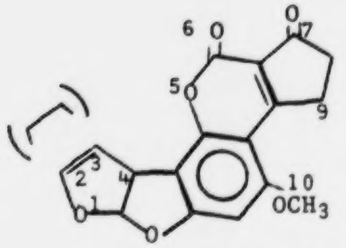
Table 9. Estrogens in Animal Feed

Estrogen	Chemical structure	Estrogenic activity compared with DES (on oral dosing)	Occurrance	Stability
Genistein		<10^{-5}	Soybeans (6 μg DES/kg)	Heat stable
Coumestrol		<35×10^{-5}	Alfalfa, soybeans; alfalfa infested with fungal pathogens (115 ppm)	Heat stable
Zearalenone		620×10^{-5}	Possibly produced on corn during storage particularly when infested with *Fusarium graminearum*	Heat stable
Diethylstilbestrol		1		

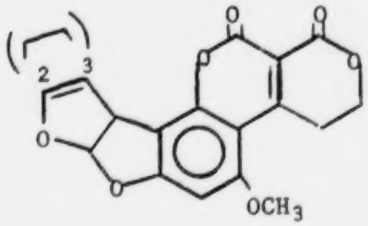
Source: Compiled from Stob (1973).

7.2.2 Aflatoxins

Aflatoxins are a family of 10 or more brightly fluorescing furanocoumarin compounds. The most prominent of these are B_1, B_2, G_1, and G_2. B_2 and G_2 are partially reduced (dihydro) forms of B_1 and G_1, respectively.



Aflatoxin $B_1(B_2)$



Aflatoxin $G_1(G_2)$

Aflatoxins are produced by Aspergillus flavus, a common mold growing on a variety of food materials including corn, soybean, peanuts, rice, and wheat. Although aflatoxin B_1 is fairly stable to heat, a reduction of aflatoxin B_1 in peanut meal from 7000 to 340 µg/kg has been achieved by autoclaving the moist meal (60% moisture content) at 15 lb pressure (120°C) for 4 h. The photodecomposition of aflatoxin is known to occur at fairly low light levels (Wilson and Hayes 1973).

Aflatoxin B_1 appears to be the most potent hepatocarcinogen known (Miller 1973). Moderate to high incidences of hepatomas and hepatocellular carcinomas have occurred in rats continuously administered aflatoxin in the diet at concentrations as low as 15 ppb. Experimental evidence suggests that aflatoxin B_1 needs to be metabolically activated to be carcinogenic in vivo (Miller 1973).

The ubiquitous presence of the mold Aspergillus flavus indicates the improbability of complete elimination of aflatoxin contamination from animal feed; however, high humidity and a temperature of 25°C have been found to be conducive to the growth of this mold. Like most fungi Aspergillus flavus is highly aerobic. The FDA has lowered the maximum amount of aflatoxins permissible in food products as methods of detection and control have improved. The upper limit has been set at 20 ppb (total, B_1, B_2, G_1, and G_2) in feeds [EPA proposed guidelines (USEPA 1979) specify 5 ppb] and 0.5 ppb aflatoxin M_1 in milk (USFDA 1980b). Aflatoxin M_1 is a metabolite of aflatoxin B_1 (4-hydroxy derivative of aflatoxin B_1) and has been identified in the milk of cows ingesting aflatoxin B_1.

7.2.3 Tricothecene Toxins

These mycotoxins comprise a family of closely related sesquiterpenoids produced by various species of fungi such as Fusarium, Myrothecium, Trichoderma, Trichothecium, Cephalosporium, Verticimonosporium and Stachybotys and are collectively called trichothecenes after tricothecin, the first isolate, discovered in 1949 by Freeman and Morrison. Fusarium tricinctum and Fusarium roseum are the two most important fungi as far as animal feeds are concerned. The former causes moldy corn (or hemorrhagic) disease and the latter causes vomiting and feed-refusal in animals. Both can grow on corn, barley, and rye. T-2 toxin, HT-2 toxin, and diacetoxyscirpenol are produced by Fusarium tricinctum, and deoxynivalenol, nivalenol, zearalenone and an unknown factor are produced by Fusarium roseum. The chemical structure, LD_{50}, natural occurrence, and biological effects of these toxins are described in Table 10.

It has been suggested by Schoental (1981) that some of the inconsistencies in epidemilogical and experimental studies on the role of various types of fats in the etiology of cardiovascular disorders and certain tumors may be related to the presence of adventitious contaminants (e.g., tricothecenes) in the fats.

Various toxicological (e.g., vomiting in ducklings), cytological (e.g., cytotoxicity to HeLa cells) and biochemical (e.g., inhibition of protein synthesis in rabbit reticulocytes) assays have been developed for tricothecenes. A sensitive radioimmunoassay capable of detecting 1 and 2.5 ppb of T-2 toxin in wheat and corn, respectively, has been recently reported (Lee and Chu 1981).

7.3 Nitrosamines

Nitrates and nitrites have been used for the preservation of fish and meat, often in conjunction with sugar, spices, polyphosphates, α-tocopherol, and sodium ascorbate. The use of ascorbic acid is rather fortuitous since it greatly reduces the chance for nitrosation of amines in the stomach by nitrous acid. Besides protecting meat from spoilage by bacteria such as Clostridium botulinum, nitrite imparts red color to the meat. It reacts with myoglobin to form nitrosylmyoglobin, which during cooking becomes denatured; the color is retained because of the formation of nitrosylmyochrome.

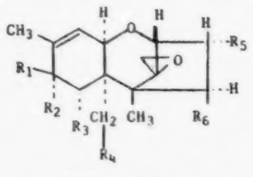
N-Nitroso compounds are produced in food principally from the reaction of naturally occurring secondary amines with nitrites that may have been added to the food or formed from bacterial reduction of nitrate. The most common volatile nitrosamine present in food is N-nitrosodimethylamine (NDMA). N-Nitrosopyrrolidine and N-nitrosomorpholine have also been detected. The latter, however, has been shown to be formed principally from morpholine used as a corrosion inhibitor in boilers when food is processed by steam in a smokehouse.

Table 10. Fusarium Toxins[a]

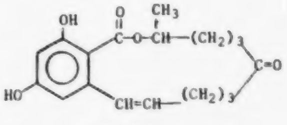
Toxin	Formula[b]	LD_{50}[c]	Foods Naturally Contaminated with Mycotoxin	Comments
Fusarium tricinctum				
Diacetoxyscirpenol	Structure I $R_1,R_2,R_3=H$ $R_4,R_6,=OAc$ $R_5=OH$	0.75 mg/kg IP(R) 7.3 mg/kg oral (R)	Millet, wheat, oats, rye, buckwheat, corn	Most potent skin irritant
T-2 toxin	Structure I $R_1,R_3=H$ $R_2=$3-methylbutyryloxy $R_4,R_6=OAc$ $R_5=OH$	4 mg/kg oral (R)	Fescue, corn, other cereals	May be associated with "fescue foot" of cattle
HT-2 toxin[e]	Structure I $R_1,R_3=H$ $R_2=$3-methylbutyryloxy $R_4=OAc$ $R_5,R_6=OH$	9 mg/kg IP(M)	Barley, corn, rye	Hemorrhagic disease
Fusarium roseum				
Nivalenol	Structure I $R_1,=O$ $R_3,R_4,R_5,R_6=OH$	4.1 mg/kg IP(M)	Rice	Suppresses DNA synthesis and protein synthesis of HeLa cells; no microbial activity
Deoxynivalenol[e] (vomitoxin)	Structure I $R_1=O$ $R_6=H$ $R_3,R_4,R_5=OH$	70 mg/kg IP(M)	Corn, wheat, pellitized feed	Coexists almost always with zearalenone[d], causes vomitting and food refusal
Zearalenone	Structure II	Unknown	Corn, hay, barley, and pelletized feed	Causes genital hypertrophy; has body growth promoting activity

[a] Adapted from Wilson and Hayes (1973) except when otherwise indicated.

[b]



STRUCTURE I



STRUCTURE II

[c] R (rat), M (mouse).
[d] Wyllie and Morehouse (1977).
[e] Ueno (1980).

Volatile nitrosamine contamination of laboratory animal diets has been investigated by Edwards et al. (1979). Low levels of N-nitrosodimethylamine (1-4 ppb) have been found in most of the commercial pelleted diets. Higher levels (5-50 ppb) of N-nitrosodimethylamine (NDMA) have been detected in three out of seven samples of NIH open formula rat and mouse ration, and low levels of N-nitrosopyrrolidine (0.3 ppb to 2.1 ppb) have also been detected in these samples. Silverman and Adams (1983) investigated the presence of N-nitrosamines in laboratory animal feed and bedding used by NIH using a gas chromatograph with a thermal analyzer. N-Nitrosodimethylamine was detected in all samples of NIH-07 meal (1.2-6.9 ppb) and 62% of NIH-07 pellets (0.6-35.2 ppb). It was also detected in 66% of closed formula meal (1.9-45.1 ppb) and 83% of closed formula pellets (0.2-21.3 ppb). Low concentrations (1-4 ppb) were found in samples of hardwood chip and corncob bedding. It appears that the poor quality of the fish meal is responsible for the higher nitrosamine content of NIH-07 diets. The elevated temperature created during the regrinding of food pellets to produce meal diets may be a contributing factor to the formation of nitrosamines. In addition, atmospheric nitrogen oxides may be absorbed and subsequently react with secondary or tertiary amines on the greater surface area of the meal diet.

It has been demonstrated that as little as 10 ppb of NDMA included in the water supply of Strain A/J mice (tumor-prone) during the whole fetal, weaning, and post-weaning period (up to 22 weeks of age) causes a tripling in the lung tumor incidence from 8% to 23% (Edwards and Fox 1979). However, it is likely that even 50 ppb of NDMA may not cause a significantly increased incidence of cancer in non-tumor-prone mice. Synergistic effects of NDMA with 3-methylcholanthrene have been demonstrated at a high NDMA level but not at the low levels found in laboratory animal diets. However, it seems advisable to keep the level of NDMA in laboratory animal diets as low as possible to avoid any potential problem.

At one time it was thought that an appreciable quantity of volatile nitrosamines present in the feed is lost during the pelletization process (Knapka 1979), but this view is now discounted (personal communication from Knapka 1982, NIH, and Shelton 1982, Ralston Purina). One commenter has reported (comments respective to proposed EPA guidelines) that autoclaving of the feed reduces the nitrosamine content. Although there appears to be no documentation of this observation in the open literature, investigating ways and means for reducing the nitrosamine content of fish meal used in the production of laboratory animal feed would certainly provide valuable information.

The detection and estimation of nitrosamines in animal feed at the ppb level is carried out using a thermal energy analyzer nitrosamine detector, interfaced with a gas or liquid chromatograph (Edwards et al. 1979).

7.4 Preservatives

Two of the most widely used preservatives in laboratory animal feed are ethoxyquin and butylated hydroxyanisole (BHA). The former is used as an antioxidant in vitamin A formulations (Shelton 1982, NIH 1980). Ethoxyquin retards the oxidation of carotene, xanthophylls, and vitamins A and E and prevents organic peroxide formation in canned pet food. It is also added to dehydrated forage prepared from alfalfa, barley, clover, bermuda grass, corn, fescue, oats, orchard grass, sorghums, wheat, etc., for this same reason. The tolerance limit for ethoxyquin in animal feed has been set at 150 ppm by the FDA (USFDA 1980a).

Vitamin A stabilized with ethoxyquin is manufactured by Hoffmann-La Roche, New Jersey. In a micronized form, it contains gelatine and other additives, as well as ethoxyquin, 7%, and vitamin A acetate, 650,000 IU/g. This calculates to 8 ppm of ethoxyquin in feed containing 75 IU of vitamin A/g (EPA proposed maximum in rodent feed, see Table 1), well below the tolerance limit set by the FDA. However, the purified diet described for rabbits in the Nuritional Requirements for Rabbits (NAS 1977) permits a rather high concentration of ethoxyquin (250 ppm).

Soybean oil contains sufficient amounts of natural antioxidants, making further addition of preservatives unnecessary; however, whenever animal fat is used in feed formulation, BHA has been frequently used as a preservative. For instance, Purina Rodent Chow #5002 does not contain animal fat and consequently no BHA. The Agway RMH 3000 diet, Purina Canine Diet #5007, Purina Guinea Pig Chow #5026, and the NIH dog diet formulation contain animal fat and unspecified amounts of BHA. There is no evidence of storage of BHA in fatty tissues in rats ingesting BHA and no evidence of chronic toxicity in rats fed up to 91% (1000 ppm) in the diet for 2 years (Patty's Industrial Hygiene and Toxicology 1981a). Both ethoxyquin and BHA have been found, however, to be potent inducers of hepatic epoxide hydratase when administered in the food of rats at 0.1% for 14 days (Kahl and Klaus 1979), potentially resulting in enhanced metabolism of a toxicant.

Appreciable improvement (20-30%) in the stability of vitamin C in pelletized feed during storage has been claimed for the ethylcellulose-coated vitamin C preparation of Hoffmann-La Roche. The NIH open formula diets specify the use of this stabilized form of vitamin C. However, the product has yet to gain popularity with feed manufacturers. Ethylcellulose presumably affords protection by acting as a moisture barrier (Hoffmann-La Roche n.d.). It is very inert physiologically and has been found to be nontoxic (Patty's Industrial Hygiene and Toxicology 1981b).

The NIH open formula diet also permits the use of propylene glycol, propyl gallate, and citric acid as preservatives in animal feed (NIH 1980); however, the allowable concentrations of these ingredients have not been specified.

7.5 Tolerance Limits of Contaminants and Preservatives in Animal Feed

Table 11 lists the tolerances proposed by EPA for certain contaminants and preservatives in rodent diets (USEPA 1979). For comparison the maximum concentrations of feed contaminants considered acceptable for natural ingredient rations for rodent diets of the National Center for Toxicological Research (NCTR) (NRC 1976), and the maximum levels of contaminant permitted (guaranteed analysis) in the Agway R-M-H (rat-mouse-hamster) 3000 diet and guinea pig diet (Agway Inc. n.d.) and in the Ralston Purina Guinea Pig Chow #5026 and Rodent Chow #5002 (Ralston Purina Co. 1980) are also provided. Also included in Table 11 are the maximum permitted contaminant levels in several nonrodent diets. When the data in this table are compared, the following becomes evident:

1. The EPA maximum tolerances exceed those of NCTR for all contaminants with the exception of estrogenic activity (1 ppb vs 2 ppb) and PCB (50 ppb vs 500 ppb).

2. The Agway R-M-H 3000 and guinea pig diets are identical with respect to maximum allowable levels of contaminants whereas the maximum permissible concentrations of malathion, DDT, cadmium, mercury, and PCB are lower in these diets than in the Purina Rodent Chow #5026.

3. The guaranteed maximum levels of contaminants permitted in commercial rodent diets exceed the proposed EPA maximum tolerance for lindane, heptachlor, DDT (Purina chows only), dieldrin, cadmium, mercury (Purina chows only), and PCB (Purina chows only).

4. No preservatives are allowed according to the proposed EPA guidelines, whereas all the commercial producers use butylated hydroxyanisole in diets containing animal fats.

Besides the contaminants listed in Table 11, the feed industry also monitors the level of heptachlor epoxide (a metabolite of heptachlor), endrin, chlordane, benzene hexachloride, toxaphene, phorate, diazinon, disulfoton, methyl (ethyl) parathion, endosulfan, ethion, and carbophenothion. The maximum levels of these contaminants permitted in Agway and Purina rodent feed are indicated in Table 12 and, as shown, are quite similar between the two producers. (Table 11 was restricted to a comparison of those contaminants listed in the proposed EPA guidelines - a list which the EPA recognizes as being inconclusive.)

In addition to the contaminants listed in Tables 11 and 12, a host of chemicals (primarily pesticides) are identified by the FDA (USFDA 1980a) and EPA (USEPA 1981) as potential contaminants in animal feeds. Table 13 lists some of the chemicals more likely to be found in laboratory animal feeds, along with their uses and tolerance levels. Neither the EPA nor the FDA tolerance listed is that for the finished feed but rather for raw commodities and feed ingredients, respectively. Pyrethrins in conjunction with piperonyl butoxide have been reported to be

Table 11. Contaminants and Preservatives in Laboratory Animal Feeds

Contaminants	EPA[b] proposed tolerance for rodent diet		National Center[e] for Toxicological Research rodent diet	Agway[c,f] (Charles River) R-M-H 3000	Purina[d] Rodent Chow® #5002	Purina[d] Guinea Pig Chow® #5026	Agway[c] (Charles River) Guinea Pig Diet	Agway[c] (Charles River) Rabbit Diet	Agway[c] (Canine Diet respond 2000	Purina[d] Rabbit Chow® #5322	Purina[d] Canine Diet #5007
	min.	max.									
Aflatoxins (total) ppb		5[g]	1.00	5.0	10.0	10.0	5.0	5.0	5.0	10.0	10.0
Estrogenic activity (DES eq), ppb		1	2.00	NG	NG	NG	NG	NG	NG	NG	NG
Lindane, ppb		20	10.00	50	50	50	50	50	50	50	50
Heptachlor, ppb		20	10.00	30	30	30	30	30	30	30	30
Malathion, ppm		2.5	0.50	0.3	0.5	0.5	0.3	0.3	0.3	0.5	0.5
DDT (total), ppb		100[h]	150.00	100	150	150	100	100	100	150	150
Dieldrin, ppb		20	10.0	30[i]	50.0	50.0	30[i]	30[i]	30[i]	50.0	50.0
Cadmium, ppb		160	50.0	200	500	500	200	200	200	500	500
Arsenic, ppm		1.0	0.25	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lead, ppm		1.5	1.00	1.5	1.5	1.5	1.5	1.5	3.0	1.5	3.0
Mercury, ppb		100	50.0	100	200	200	100	100	100	200	200
Selenium, ppm	0.1	0.6	0.5	0.2	NG	NG	0.2	0.2	0.2	NG	NG
PCB, ppb		50	500.00	50	150	150	50	50	50	150	150
Nitrosamines, ppb		10	NG	NG	5-12[j]	NG	NG	NG	NG	NG	NG
Antibiotics		0	NG	NG	NG	NG	NG	NG	NG	NG	NG
Preservative		0	NG	BHA+[k]	NG	BHA+[k]	BHA+[k]	NG	BHA+[k]	NG	BHA+[k]

NG = Not given.

[a] All data are maximum allowable levels unless otherwise stated.

[b] Data taken from USEPA (1979).

[c] Data taken Agway Inc. (n.d.).

[d] Data taken from Ralston Purina Company (1980).

[e] Data taken from NRC (1976).

[f] Rat-Mouse-Hamster diet.

[g] Animal exposure to aflatoxins in food is limited to the level of 20 ppb (total B_1, B_2, G_1, G_2) under the current FDA regulations (USFDA, 1981).

[h] The permissible limit in animal feed is 5 ppm under the USFDA (1980).

[i] +Aldrin.

[j] Personal communication from Purina.

[k] BHA, butylated hydroxyanisole.

Table 12. Pesticides and Contaminants in Laboratory Animal Feeds

Common name	Chemical name	Use	Tolerance, ppm[a] Agway (Charles River) R-M-H 3000[b]	 Purina rodent chow® #5002[c]
Aldrin + dieldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-exo-1,4-endo-5,8-dimethanonaphthalene (aldrin)	Insecticide (soil insects)	0.03	0.05+0.05
Heptachlorepoxide		Metabolite of heptachlor	0.03	0.05
Endrin	Hexachloroepoxyoctahydro-endo, endo-dimethanonaphthalene	Insecticide	0.03	0.05
Chlordane	1,2,4,5,6,7,8,8-Octachlor-2,3,3a,4,7,7a-hexahydro-4,7-methanoindane	Insecticide (termites)	0.05	0.05
BHC	Benzene hexachloride	No longer produced or sold for domestic use in the United States	0.05	NG
Toxaphene	Chlorinated camphene	Insectcide (soybeans, sorghum, and peanuts)	0.2	NG
Phorate, Thimet	O,O-Diethyl S-{(ethylthio)-methyl}phosphorodithioate	Insecticide (alfalfa, corn, wheat, etc.)	0.3	0.5
Diazinon	O,O-Diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate	Insecticide and pesticide	0.3	0.5
Disulfoton	O,O-Diethyl S-{2-(ethylthio)-ethyl}phosphorodithioate	Insecticide and acaricide	0.3	0.5
Methyl(ethyl)parathion	O,O-Dimethyl)ethyl) o-p-nitrophenyl phosphorothioate	Insecticide	0.3	0.5+0.5
Endosulfan, Thiodan	6,7,8,9,10,10-Hexachloro 1,5,5a,6,9,9a-hexahydro-6,9-methano-1,4,3-benzo(e)-dioxathiepin-3-oxide	Insecticide	0.3	0.5
Ethion	O,O,O,O-Tetraethyl S,S-methylene bisphosphorodithioate	Insecticide	0.3	0.5
Carbophenothion, Trithion	S-{(p-chlorophenylthio)methyl} O,O-diethyl phosphorodithioate	Acaricide	0.3	0.5

NG = Not given.

[a] Values are maximum allowable levels.

[b] Data taken from Agway (n.d.). Rat-Mouse-Hamster 3000.

[c] Data taken from Ralston Purina Company (1980).

Table 13. Pesticides in Laboratory Animal Feeds

Common name	Chemical name	Use	Feed	Tolerance (ppm)	
				USFDA[a]	USEPA[b]
Acephate	O,S-Dimethyl acetylphosphoramidothioare	Insecticide	Soybean	4[c]	1.0
Aldicarb	2-Methyl-2(methylthio)proionaldehyde O-(methyl-carbamoyl)oxime	Systemic insecticides, acaricide, nematicide for soil use	Cottonseed hulls	0.3	
			Soybean		0.02
Aluminum phosphide	Aluminum phosphide	Fumigant insecticide	Bulk grain	0.1[e]	0.01
NF	4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5 (4H)-one	Herbicide	Barley, wheat	3.0	0.75
			Sugar cane molasses	0.3	NG
			Tomato pomace	2.0	NG
Banvel	2-Methoxy-3,6-dichlorobenzoic acid or 3,6-dichloro-*o*-anisic acid	Herbicide	Corn, wheat, sorghum	—[h]	—[h]
Benomyl	Methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate	Fungicide	Dehydrated citrus pulp	50.0	
			Citrus fruit		10.0
Bentazon, Basagran	3-(1-Methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide	Herbicide	Soybean	NA	0.05
Butachlor	2-Chloro-2',6'-diethyl-N-(butoxymethyl)acetanilide	Herbicide	Rice, bran	0.5	NG
Chlordimeform	N'-(4-Chloro-o-tolyl)-N,N-dimethylformamidine	Insecticide	Cottonseed hulls	10.0	
			Cottonseed		5.0
Chlorpyrifos	O,O-Diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	Insecticide	Sugar beet molasses	3.0	NG
			Sugar beet pulp, dried	11.0	NG
			Sorghum milling fractions	1.5	
			Sorghum grain		0.75
2,4-D	2,4-Dichloro-phenoxy acetic acid	Herbicide	Barley, oats, rye, wheat	2.0	0.5
			Sugarcane molasses	5	
Dalapon	2,2-Dichloropropionic acid	Growth regulation	Dehydrated citr pulp	20.0	
			Citrus frut, grain crops (excl. wheat)		0.5
Daminozide, Alar	Butanedioic acid mono (2,2-dimethyl hydrazide)	Plant regulator	Dried tomato pomace	600.0	
			Peanut	90.0[d]	30.0
Demeton, systox	Mixture of O,O-diethyl O-{2-(ethylthio)ethyl} phosphorothiate and O,O-diethyl S-{2-(ethylthio)ethyl} phosphorothioate	Systemic insecticide	Sugar beet pulp	5.0	
			Sugar beet		0.5
			Dehydrated citrus pulp	5.0	
			Citrus fruit		0.75
Dialifor, Torak	S-(2-Chloro-1-phthalimidoethyl) O,O-diethyl phosphorodithioate	Insecticide	Dehydrated citrus pulp	15.0	
			Citrus fruit		3.0
NF	N'-(2,4-Dimethylphenyl)-N-[{(2,4-dimethylphenyl) imino}methyl]-N-methylmethanimidamie	Insecticide	Dehydrated citrus pulp	3.0	
NF	2-Ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate	Herbicide	Sugar beet molasses	0.5	
			Sugar beet		0.3
NF	S-[2-(Ethylsulfinyl)ethyl] O,O-dimethyl phosphorothioate	Insecticide	Sorghum	2.0	NG
NF	O-Ethyl O-[4-(methylthio)-phenyl] S-propyl phosphorodithioate	Insecticide	Cottonseed hulls	1.0	
			Cottonseed		0.5

Table 13. (continued)

Common name	Chemical name	Use	Feed	Tolerance (ppm) USFDA[a]	Tolerance (ppm) USEPA[b]
NF	Hexakis (2-methyl-2-phenylpropyl) distannoxane	Insecticide	Dehydrated citrus pulp	7.0	
			Citrust fruit		4.0
Magnesium phosphide	Magnesium phosphide	Fumigant insecticide	Not specified	0.1^e	
			Barley, corn, cottenseed		0.1
Methanearsonic acid	Methanearsonic acid	Herbicide	Cottonseed hulls	0.9^f	
			Cottonseed		0.7
			Sugarcane mollasses	3.0^f	
Paraquat	1,1'-Dimethyl-4,4'-bipyridinium dichloride	Herbicide	Sunflower seed hulls	6.0^g	
			Sunflower seed		2.0
Phosalone, Zolone	O,O-Diethyl S-[-(6-chloro-2-oxo-benzoxazolin-3yl)-methyl]phosphorodithioate	Insecticide	Dehydrated citrus pulp	12.0	
			Citrus fruit		3.0
Picloram, Tordon	4-Amino-3,5,6-trichloropicolinic acid	Herbicide	Barley, oats, wheat	3.0	0.5-1.0
Piperonyl butoxide	α-[2-(2-l-Butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene	Synergist wih pyrethrin (insecticide); treatment of bags used for packaging food	Not specified	10.0	
			Barley, rye, wheat		20.0
Plictran	Tricyclohexyl hydroxystannane	Acaricide	Dried citrus pulp	8.0	
			Citrus fruit		2.0
Profenofos, Curacron	O-(4-Bromo-2-chlorophenyl)-O-ethyl S-propyl phosphorothioate	Insecticide	Soybean	—[h]	NG
			Cottonseed hulls	6.0	NG
Propanil	3',4'-Dichloropropionanilide	Herbicide	Rice milling fraction	10.0	
			Rice		2.0
Pyrethrins	—	Insecticide; treatment of bags used for packaging food	Not specified	1.0	
			Barley, rye, wheat		3.0
Simazine	2-Chloro-4,6-bis(ethylamino)-s-triazine	Herbicide	Sugarcane molasses	1.0	
			Alfalfa		15.0
TDE (DDD)	1,1-Dichloro-2,2-bis(p-chlorophenyl)ethane	Insecticide	Dried tomato pomace	100.0	
			Tomato		7.0
NF	S,S,S-Tributyl phosphorotrithioate	Herbicide	Cottonseed hulls	6.0	
			Cottonseed		0.25
Tutane	Sec-butylamine	Fungicide	Dehydrated cirus pulp	90.0	
			Citrus fruit		30.0
Zn ion + maneb		Fungicide	Barley, oats, rye, wheat	20.0	
			Wheat		1.0
			Corn grain		0.1

NA = not applicable; NF = not found; NG = not given.

[a] Tolerances in feed ingredients, USFDA (1980).

[b] Tolerances in raw commodities, USEPA (1981).

[c] Soybean meal.

[d] Peanut meal.

[e] PH_3.

[f] As_2O_3.

[g] Cation.

[h] No tolerance given.

[i] Use indicated in Farm Chemicals Handbook (1981).

used for the treatment of bags used for packaging, primarily to discourage insect infestation of the stored food (USFDA 1980a). The current manufacturing practice probably does not make use of any insecticide treatment of the bags used for packaging animal feed, as exemplified by the Ralston Purina Company (Jon Ford 1982).

7.5.1 Considerations for Establishing Tolerances for Contaminants

Three of the most important considerations in setting tolerance limits for contaminants in animal feed are analytical capability, biological no-effect levels, and interactions between dietary components and contaminants. There is no point in setting a tolerance limit below the detection limits of the currently available analytical techniques. Normal screening levels of detection (not necessarily the detection limit) of different contaminants have been reported by Greenman et al. (1980) (see Table 14). Assay of contaminants at such low levels provides a challenge to the analytical chemist. Therefore, reproducibility of various assays and variation from laboratory to laboratory need to be investigated. The open literature contains biological effect and no-effect data for some contaminants (see Tables 15 and 16). Such data need to be developed for other contaminants of interest. Interaction between dietary components and toxicants is an active area of research. A few representative examples are given in Table 17. Many dietary constituents such as BHA are known to affect the metabolism of chemical carcinogens by microsomal mixed-function oxidases in liver, lung, intestine, and other tissues (Schrauzer 1977). Any synergism, antagonism or initiator-promotor activity of the contaminant is certain to influence the outcome of long-term toxicity studies.

7.5.2 Suggested Changes in the Tolerance Limits for the Contaminants Listed in the EPA GLP

7.5.2.1 Aflatoxins

In harmony with the current FDA guidelines (USFDA 1980b), consideration should be given for setting a tolerance of 0-20 ppb.

7.5.2.2 Estrogenic activity

This contaminant has not posed any serious problem in the past so far as animal feed manufacture is concerned (Greenman et al. 1980). The estrogenic activity has been found to be so low that it is no longer monitored at NIH (personal communication from J. Knapka 1980). The NIH bioassay procedure uses 4 ppb of DES (diethylstilbestrol) as a positive control, and animal feeds are regarded to have positive estrogenic activity only when they exhibit higher activity than the standard containing 4 ppb of DES. The tolerance level possibly should be raised to 4 ppb.

7.5.2.3 Lindane

Even though a concentration of 0-50 ppb of lindane is allowed in animal feeds by many producers, most of the commercial feed analyzed by

Table 14. Normal Screening Levels of Detection

Analyte	Normal screening level of detection	Reference
Aflatoxin	5 ppb	Horwitz (1970c) Stubblefield et al. (1967) Beljaars et al. (1975) Przybylski (1975)
Pesticides and PCBs		
DDT	5 ppb	Bowman and Beroza (1970)
Dieldrin	3 ppb	Bowman et al. (1971)
Heptachlor	3 ppb	McMahon and Sawyer (1975a, 1975b)
Lindane	3 ppb	Horwitz (1970d)
PCBs	20 ppb	Burchfield and Johnson (1965)
Malathion	50 ppb	Brody and Chaney (1966)
As	50 ppb	Hundley and Underwood (1970) Horwitz (1970b)
Cd	5 ppb	J. Assoc. Off. Anal. Chem. (1973a)
Ca	1000 ppb	Heckman (1968) Horwitz (1970a) Horwitz (1970b)
Pb	500 ppb	Horwitz (1970b) J. Assoc. Off. Anal. Chem. (1973b)
Se	40 ppb	Hoffman et al. (1968)
Hg	20 ppb	J. Assoc. Off. Anal. Chem. (1971)
Cu	20 ppb	Horwitz (1970a)
Zn	20 ppb	Horwitz (1970a) Heckman (1968)
Vitamin A	10 IU/g	Bowman and King (1973)
Vitamin B_1	5 ppm	Bowman (1973)
Fat	0.5%	Horwitz (1970a)
Protein	0.5%	APHA (1965) USEPA (1969)

Source: Adapted from Greenman et al. (1980), p. 237.

Table 15. Biologically Effective Concentrations of Selected Organic Feed Contaminants

			Dietary concentration (ppm)		
Compound	Species	End point	Effect	No effect	Reference
Aflatoxin	Mouse	Liver lipids	0.114	0.057	Lindenfelser et al. (1976)
		Liver tumors	1	-	Newberne and Butler (1969)
	Rat	Liver hyperplasia	0.001	-	Wogan et al. (1974)
		Liver tumors	0.015	-	Wogan and Newberne (1967)
DDT	Mouse	Reproduction		3	Tarjan and Kemeny (1969)
		Neoplasia	3		Tarjan and Kemeny (1969)
		Liver tumors	2	-	Tomatis et al. (1972)
		Liver tumors	250	20	Terracini et al. (1973)
		Lung tumors	10	-	Shabad et al. (1973)
	Rat	Liver morphology	5	1	Laug et al. (1950)
		Microsomal enzymes	2.5	2	Gillett (1968)
		Microsomal enzymes	1	0.2	Kinoshita et al. (1966)
Dieldrin	Mouse	Reproduction	2.5	-	Virgo and Bellward (1975)
		Mortality	10	1	Walker et al. (1972)
		Liver tumors	0.1	-	Walker et al. (1972)
	Rat	Microsomal enzymes	5	1	Gillett and Chan (1968)
		Microsomal enzymes	5	2	Den Tonkelaar and Van Esch (1974)
		Liver weight	1	0.1	Walker et al. (1969)
		Brain lesions	0.34	0.02	Harr et al. (1970)
Heptachlor	Mouse	Liver weight	1	-	Epstein (1976)
		Liver tumors	5	1	Epstein (1976)
	Rat	Microsomal enzymes	5	1	Gillett and Chan (1968)
		Microsomal enzymes	2	-	Den Tonkelaar and Van Esch (1974)
Lindane	Mouse	Tumor induction and liver morphology		50	Weisse and Herbst (1977)
	Rat	Microsomal enzymes	50	20	Den Tonkelaar and Van Esch (1974)
		Microsomal enzymes and liver weight	20	2	Pelissier and Albrecht (1976)
Malathion	Rat	Liver weight	100	-	Cecil et al. (1974)
		Cholinesterase	1000	100	Hazelton and Holland (1953)
		Cholinesterase and EEG	380[a]	-	Desi et al. (1976)
		DMBA-induced tumors	250	-	Silinskas and Okey (1975)

[a] Estimated equivalent.

Source: Greenman et al. (1980), p. 242.

Table 16. Biologically Effective Concentrations of Selected Heavy Metals

Element	Species	End point	Dietary or water concentration (ppm)		Reference
			Effect	No effect	
As	Mouse	Reproduction	5	-	Schroeder and Mitchener (1971a)
		Growth and mortality	-	5	Schroeder and Mitchener (1971a)
		Mortality	5	-	Schroeder and Balassa (1967)
		Mammary tumors[a]	10	-	Schrauzer and Ishmael (1974)
	Rat	Growth	62.5[b]	31.25[b]	Bryon et al. (1967)
Cd	Mouse	Reproduction	10	-	Schroeder and Mitchener (1971a)
		Mortality	5	-	Schroeder et al. (1964)
	Rat	Renal vasculature	0.2	-	Fowler et al. (1975)
		Hypertension	1	-	Perry and Erlanger (1974)
Pb	Mouse	Mortality	5	-	Schroeder et al. (1964)
		Growth	-	5	Schroeder and Mitchener (1971a)
		Antibody formation	14	-	Koller and Kovacic (1974)
	Rat	δ-Aminolevulinate dehydratase	10	1	Hubermont et al. (1976)
		Mortality	5	-	Schroeder et al. (1965)
Hg	Mouse	Mortality and growth	5	-	Schroeder and Mitchener (1971a)
	Rat	Hypertension	5	2.5	Perry and Erlanger (1974)
		Behavior and learning	2[b]	-	Olson and Boush (1975)
		Kidney ultrastructure	2[b]	-	Fowler (1972)
Se	Mouse	Reproduction	3	-	Schroeder and Mitchener (1971a)
		Growth and mortality	3	-	Schroeder and Mitchener (1972)
		Mammary tumors[c]	2	-	Schrauzer and Ishmael (1974)
	Rat	Growth, mortality, and tumors	2	-	Schroeder and Mitchener (1971b)
		AAF-induced tumors[d]	0.5[b]	0.1[b]	Harr et al. (1973)
		Hepatitis	2.5[b]	0.5[b]	Harr et al. (1973)

[a] Decreased incidence, increased tumor growth rate.

[b] Mixed in the diet; all others were administered in water.

[c] Decreased tumor incidence.

[d] Increased latent period.

Source: Greenman et al. (1980), p. 241.

Table 17. Interaction of Dietary Factors with Toxicants

Dietary factors	Toxic agents	Biological response	Reference
Protein			
Deficiency	Carbon tetrachloride Dimethylnitrosamine	Reduced toxicity (rats)	McLean and Verschurn (1969)
	Pesticides	Increased toxicity	Boyd (1969)
	Mercuric chloride	Reduced toxicity (rats)	Swithin and Yagi (1958)
	Dimethylnitrosamine	Reduced carcinogenicity	Swan and McLean (1968)
	Dimethylnitrosamine	Reduction in spontaneous hepatomas (rats)	Silerstone and Tannenbaum (1951)
Fat			
Increased intake	Chloroform	Increased sensitivity	Goldschmidt et al (1939)
Addition of herring oil and linoleic acid		Maximum synthesis of cytochrome P-450	Marshall and McLean (1969)
High fat, low in choline, methionine, folic acid	Carcinogens	Enhanced liver tumor induction	Rogers et al. (1974), Rogers (1975)
High fat	7,12-Dimethylbenzanthracene	More mammary tumors (rats) than in animals on regular diets	Corrou and Khor (1970)
Lipotropes			
Low lipotropes	Azaserine	Increased susceptibility to liver carcinogenesis	Shinozuka et al (1978)
Low lipotropes	Aflatoxin	Increased hepatotoxicity	Newberne et al (1968)
Low lipotropes, high fat	7,12-Dimethylbenzanthracene	More mammary tumors (rats) than in animals on regular diets	Newberne and Rogers (1976)
Low lipotropes, high fat	N-2-fluorenylacetamide	More mammary tumors (rats) than in animals on regular diets	Newberne and Rogers (1976)
Vitamins			
Vitamin A	Polynuclear aromatic hydrocarbons	Inhibits induction of squamous metaplasia and squamous cell tumors	Saffiotti et al (1967) Cone and Nettesheim, 1973
Vitamin A	Benzo(a)pyrene	Enhanced induction of respiratory tract tumors	Smith et al (1975) Smith, Rogers, and Newberne (1975)
Ascorbic acid	Secondary amines	Reduces nitrosation	Fan (1973)
α-Tocopherol (Vit. E)	3-Methylcholanthrene	Reduces sarcomas in mice	Haber (1962)

Table 17. (continued)

Dietary factors	Toxic agents	Biological response	Reference
Minerals			
Arsenic	—[a]	Decreases cancer susceptibility; an essential micronutrient?	Frost (1978)
High Zinc	Molybdenum (Mo)	Increased toxicity to Mo (rats)	Brinkman and Miller (1961)
Selenium	Cadmium (Cd), Mercury (Hg)	Decreased toxicity to Cd, Hg	Farkas (1978)
Selenium	Carbon tetrachloride (CCl_4)	Reduces the toxicity of CCl_4 which catalyzes lipid peroxidation.	Fodor and Kemeny (1965)
Low iron	—[a]	Increased hepatic mixed function oxidase activity	Becking (1976)
Low magnesium, potassium	—[a]	Decreased oxidase activity	Becking (1976)
Butylated hydroxyanisole, butylated hydroxy toluene	Benzo(a)pyrene (BP)	Decreases the carcinogenicity of BP	Carr (1982)
High fiber diet	Aflatoxin, estrogens	Reduces the toxicity and carcinogenicity of the agents	Carr (1982)
Fiber	Carcinogen/procarcinogens	Decreases transit time, alters the bacterial flora in the gut, reduces incidence of colon cancer	Burkitt (1971, 1976)
Butylated hydroxyanisole, ethoxyquin	Benzo(a)pyrene (BP) 7,12-Dimethylbenzanthracene Dimethylnitrosamine	Decreases the carcinogenicity of BP	Wattenberg (1979)

[a] No toxicant interaction.

Greenman et al. (1980) during 1974-78 showed much less than 20 ppb (see Table 18). Feed manufacturers should be able to comply with the currently set level of 20 ppb. No change, therefore, seems warranted.

7.5.2.4 Malathion

Consideration should be given to reducing the tolerance level of malathion from 2.5 ppm to perhaps 0.5 ppm since it appears the feed industry can easily comply (see Tables 11, 18).

7.5.2.5 Cadmium

Commercial samples (see Table 18) contained much less than the proposed tolerance of 160 ppb of Cd during 1974-78 (Greenman et al. 1980). However, it should be noted that the Agway and Ralston Purina certified diets may contain 200 ppb and 500 ppb, respectively (see Table 10). If there is any problem meeting this tolerance level (160 ppb), Cd-rich ingredients in the feed should be identified and replaced. No change seems warranted.

7.5.2.6 Arsenic

Until the status of As as a micronutrient is established, the current tolerance limit of 1.0 ppm seems appropriate (see Sect. 10.2 for further discussion).

7.5.2.7 Selenium

Consideration should be given to reducing the upper level of Se to 0.4 ppm, since Se at a 0.5 ppm level has been reported to increase the latent period for 2-acetylaminofluorene-induced tumors (Greenman et al. 1980) (see Sect. 10.3 for further discussion).

7.5.2.8 Preservatives

Addition of ethoxyquin, ethylcellulose, and butylated hydroxyanisole (BHA), should perhaps be permitted in the feed in keeping with the current practice for the preservation of vitamin A, vitamin C and animal fat, respectively. This will also permit an increase in the shelf life of the feed to six months instead of three months as currently stipulated in the EPA GLP (USEPA 1979).

Hoffman-La Roche formulations of protected vitamin A contain 7% of ethoxyquin; at the highest level of vitamin A permissible in the feed (75 IU/g) in the proposed EPA guidelines (USEPA 1979), the concentration of ethoxyquin is 8 ppm. This is well below the tolerance limit set by the FDA (150 ppm). A tolerance limit of 10 ppm for ethoxyquin in animal feed would seem satisfactory.

The amount of crude fat used in animal diet formulations can be expected to be as high as 9% (see Table 1). USFDA (1980a) allows a BHA

Table 18. Results of Analyses of Laboratory Animal Diets

Analyte[a]	Annual average[b]					Accumulated 5-yr value				
	1974 (*n*=14)	1975 (*n*=15)	1976 (*n*=54)	1977 (*n*=34)	1978 (*n*=31)	Mean (*n*=148[c])	SD	Maximum value	Minimum value	CV[d]
Lindane (ppb)	2.20	5.96	1.81	0.84	0.26	1.67	3.6	40	0.02	215
Heptachlor (ppb)	2.64	4.10	1.11	0.32	0.02	1.07	2.24	12.3	0.02	209
Malathion (ppm)	0.11	0.38	0.62	0.10	0.10	0.33	0.53	2.4	0.005	161
DDT (ppb)	14.0	151.61	13.93	18.10	10.11	27.7	48.4	309.1	0.15	175
PCB (ppb)	0.5	0.5	0.5	19.85	16.80	8.7	15.0	55.0	0.5	172
Dieldrin (ppb)	4.9	12.15	1.21	1.43	0.18	2.35	4.6	26.3	0.08	197
Cd (ppb)	79.4	84.93	86.30	93.15	87.27	87.3	33.2	168.0	5.0	38
As (ppm)	0.038	0.01	0.38	0.32	0.01	0.25	0.28	0.92	0.01	112
Pb (ppm)	0.39	0.89	0.42	0.34	0.55	0.47	0.38	1.92	0.02	81
Hg (ppm)	0.036	0.037	0.023	0.019	0.02	0.024	0.018	0.16	0.007	75
Se (ppm)	0.30	0.43	0.42	0.22	0.30	0.34	0.15	0.66	0.04	44
Ca (%)	-	-	1.25	1.16	1.08	1.16	0.18	1.67	0.82	16
Cu (ppm)	-	-	12.99	15.20	16.72	15.0	2.8	22.0	6.9	19
Zn (ppm)	-	-	108.07	106.66	110.10	108.2	9.7	140	78	9
Vitamin A (IU/g)	36.95	27.68	49.08	40.31	38.73	41.6	36.9	439	11.5	89
Vitamin B_1 (mg/100 g)	7.36	8.91	9.03	9.46	9.61	9.1	1.25	13.1	5.7	14
Protein (%)	-	-	24.43	23.80	24.50	24.2	2.4	44.4	21.7	10
Fat (%)	-	-	5.33	5.72	5.5	5.54	0.58	6.6	4.0	10

[a] In addition to the analyses reported here, each lot was analyzed for aflatoxin and estrogenic activity; the results of these analyses were always less than the detectable concentration (5 ppb).

[b] Five new analytes were added to the surveillance list in 1976; this accounts for the voids (indicated by dashes) in the table.

[c] Results are reported for 148 production lots of autoclavable laboraory diet.

[d] Coefficient of variation.

Source: Greenman et al (1980), p. 240.

level in the diet of 0.02% of the fat content. Therefore, consideration for a tolerance limit for BHA of 20 ppm in the finished diet should be given.

Both BHA and ethoxyquin have been shown to be good inducers of enzymes and inhibit the carcinogenic effect of B(a)P and DMBA; however, the level of the preservatives used in these experiments (Wattenberg 1972) is much higher (5-10 mg/g of feed) than what would be added to animal feed to prolong its shelf life and will therefore probably not result in any significant inhibition of carcinogenesis.

7.5.3 Inconsistencies in EPA and FDA Tolerance Limits for Contaminants

A couple of inconsistencies apparently exist between the EPA and FDA tolerance limits for aflatoxin and DDT. The EPA Good Laboratory Practice Standards (USEPA 1979) specifies a maximum limitation of 5 ppb for aflatoxin (B_1, B_2, G_1, and G_2) in animal feed, whereas the Food and Drug Administration Compliance Guide (USFDA 1980b) recommends legal action if the aflatoxin level in the finished feed ingredients is above 20 ppb. The EPA Good Laboratory Practice Standards (USEPA 1979) also specifies a maximum limitation of 100 ppb for DDT (total) in animal feed, whereas the FDA (USFDA 1980a) revised as of April 1, 1980, states:

> The following tolerance is established for residues of DDT resulting from use of DDT as a pesticide on the growing agricultural crop: 100 parts per million in or on dried tomato pomace to be used in dog and cat food at levels up to 5 percent by weight of the prepared food. If the residues of TDE (DDD) on tomatoes are also present, the total of both such chlorinated compounds shall not exceed 100 parts per million.

This calculates to be 5 ppm of DDT (total) in the prepared feed, a level 50-fold higher than the limit (100 ppb) proposed in the EPA Good Laboratory Practice Standards (USEPA 1979). This is, however, in contrast with an action level of 0.5 ppm for residues of DDT, DDE, and TDE individually or in combination in processed animal feed prescribed in the FDA Compliance Policy Guide 7126.27, Attachment D (USFDA 1981).

8. MONITORING AND ANALYSIS OF CONTAMINANTS IN FEED

To insist that the industry analyze each batch of manufactured feed for the potential contaminants identified in Sect. 7 will put an almost unbearable and expensive analytical burden on them. On the other hand, investigators sometimes assume that because control animals receive the same basic diet as experimental animals, variations in nutrients or contaminants will not have significant effect on the experimental outcome. Such an assumption may not always be correct since dietary variables can have significant interactions with experimental variables or parameters. The necessity of monitoring the contaminants in animal diets has been addressed in an important paper by Greenman et al. (1980). These authors analyzed commercial rodent feed for a series of nutrients and potential contaminants during a 5-year period. Frequently, Se was found at concentrations at which it has been shown to interact with the process of chemical carcinogenesis. DDT, dieldrin, Cd, and Pb were occasionally close to concentrations known to have biological effects. Besides the seasonal and regional variation in the contaminant level of the feed crop, there may also be variation from year to year as revealed in this study. For instance, the DDT concentration in the commercial animal feed was highest in 1975 (152 ppb), whereas it was between 10-18 ppb in 1974 and in 1976-78. These considerations suggest that periodic monitoring of the contaminants in the feed ingredients and the finished feed by the manufacturer and the investigator are necessary to provide adequate quality assurance as specified in USEPA (1979). Current awareness of the farming practice and the environmental regulations should provide clues to the manufacturer as to the nature of the contaminants for which he should analyze. For example, after DDT was banned, its concentration in the animal diet has gone down, and with increasing use of unleaded gasoline in cars, the concentration of Pb in the environment has been reduced, with consequent reduction of the level of Pb in human blood (Sterba 1982). Similar reduction in the Pb level in food crops can probably be expected.

As previously mentioned, there is no point in setting tolerance limits for contaminants at a level below the analytical capability for detection and assay of the contaminants. In many cases the detection limits and accuracy of analysis are unknown. The development of new analytical methods and refinement of the existing methods for assay of contaminants at ppm/ppb levels should continue to be pursued by the scientific community. The variability in the results of the assay methods widely adopted in commercial and research laboratories should be investigated by collaborative studies as described in AOAC (1980). While no effort was made to include a comprehensive coverage of the literature for different analytical methods for contaminant analysis, the methods in use at the NIH laboratories are briefly described in Appendix A, and pertinent information concerning the analytical methods currently in use at Ralston Purina Company Laboratories at St. Louis is given in Table 19.

Table 19. References to the Methods of Analysis of Contaminants in Animal Feed and Relevant Statistical Data

Contaminant	Matrix	Average recovery	Mean level of analyte	Standard deviation	Coefficient of variation	Confidence level/ sample wt.	References for analytical methodology
Pb	Alfalfa	86.7%	2.98 ppm	0.29 ppm	9.76%	0.1 ppm/10 g	a
	Cereal	107.4%	1.41 ppm	0.14 ppm	9.65%		
Cd	Feed	93.2%	0.465 ppm	0.032 ppm	6.89%	0.05 ppm/10 g, 0.02 ppm/25 g	b
Hg	Feed		131 ppb	8 ppb	6.4%	50 ppb/1.0 g	c
Se	Feed		1 ppm	0.12 ppm	11%	0.05 ppm/1.0 g	d
As	Feed		0.03%	0.002%	5%	0.2 ppm/1.0 g	e
Aldrin, Heptachlor, Dieldrin, DDE, DDT, Lindane, Chlordane, Endrin, BHC, HCB, Mirex, Methoxychlor	Feed		-	-	-	0.02 ppm/10 g	f
PCB	Feed		-	-	-	0.15 ppm/10 g	f
Thimet, Diazinon, Disulfoton, Methyl Parathion, Malathion, Parathion, Thiodan, Ethion, Trithion	Feed		-	-	-	0.02 ppm/10 g	g
Aflatoxin	Feed		-	-	-	20 ppm/50 g	h

[a] AOAC (1980), 13th ed., 25.068-25.073.

[b] AOAC (1980), 13th ed., 2.109-2.113, 7.096-7.098, 33.089-33.094; Perkin-Elmer; Analytical Methods for Absorption Spectrophotometry, 3/73.

[c] Thorpe, Determination of Mercury in Food Products, Michigan Department of Agriculture (1970), modified.

[d] AOAC (1975) 12th ed., 3.078; Watkinson, J.H., Anal. Chem. *38*(1). Fluorometric Determination of Se in Biological Material with 2,3-Diaminonaphthalene.; Haddad, P.R. and L.E. Smythe, Talanta, *24*, 859, 1974.

[e] Monior, Williams G.W., Trace Elements in Foods, p. 213, John Wiley and Sons, Inc., NY (1949); Branford, F., Poisons (1951) 3rd ed., p. 73, The Blakeston Co., Philadelphia, Pa.; Sandell, E.B., Colorimetric Determination of Traces of Metals, p. 142, Interscience Publishers, New York, N.Y., 1974.

[f] Pesticide Analytical Manual, Vol. I, Sec. 211.13-211.17, 212.101-212.136; AOAC (1975), 12th ed., 29.001-29.018.

[g] Pesticide Analytical Manual, Vol. I & II; AOAC (1975), 12th ed., 29.001-29.018.

[h] AOAC (1975), 12th ed., Supplement Method 26.A01-26.A08.

Source: Ralston Purina Company (1982).

9. INTERACTION OF DIETARY COMPONENTS

Certain components in the laboratory animal diet are known to interact with each other. Awareness of this aspect of animal nutrition is essential for the successful formulation of animal diets. The interplay among Zn, Se, As, vitamins, amino acids, fatty acids, and phytate is described below, as is the importance of Ca:P and K:Na ratios in the animal diet and the enzyme induction by dietary constituents and contaminants.

9.1 Interplay Among Zn, Se, As, Vitamins, Amino Acids, Fatty Acids, and Phytate

Zn plays a role in the metabolism of vitamin A (Smith et al. 1976), and Zn deficiency causes alteration in fatty acid patterns in the skin of rats under essential fatty acid deficiency conditions (Betteger et al. 1979). Phytate present in soy protein interferes with the absorption of Zn; therefore, when soy protein is used as the main source of protein in animal diets the Zn level is usually increased. This is the rationale for the higher Zn content of the AIN-76^{TM} diet of 30 ppm (AIN 1977) compared with the NAS-NRC recommendation of 12 ppm for the rat diet (NAS 1978).

Selenium possesses anticarcinogenic properties at low levels in animals and humans (Schrauzer 1977). The uptake of Se can be diminished by Zn, and diets rich in sulfur-containing amino acids diminish the physiological activity or availability of Se (Schrauzer 1977). Arsenic behaves as an antagonist to Se in that it increases the urinary excretion of Se in rats and causes a deficiency of Se (Schrauzer 1977). There is also an interrelationship between Se and vitamin E. Supplements of Se can prevent or cure liver necrosis in the rat and oxidative diathesis in the chick, which are manifestations of vitamin E deficiency. Selenium, however, is ineffective against other manifestations of vitamin E deficiency such as testicular and embryonic degeneration in the rat and encephalomalacia in the chick. Sulfur-containing amino acids have a sparing effect on the vitamin E requirement in certain circumstances and offer at least partial protection against liver necrosis and oxidative diathesis (Clark et al. 1977).

9.2 Ca:P Ratio

The Ca:P ratio is important in animal nutrition and should be maintained between 1.0 and 1.5 (NAS 1978). As it has been pointed out by one commenter (comments on the proposed EPA guidelines), this ratio is more important than the absolute amounts of Ca and P in the diet. This fact should possibly be emphasized in the EPA GLP (USEPA 1979).

9.3 K:Na Ratio

The K:Na ratio varies from a low of 1.69 in Purina canine diet #5007 to as high as 7.20 in the NAS-NRC recommended diet for the rat (Tables 1, 2, respectively). The protective effect of K in rats during high Na intake in the diet has been reported (Meneely 1973). The role of the K:Na ratio in animal diets should be investigated particularly in view of the recent controversy of the value of a low sodium diet in reducing hypertension (Kolata 1982).

9.4 Enzyme Induction and Inhibition of Neoplasia by Minor Dietary Constituents, Feed Additives, and Contaminants

A few representative examples of interaction between dietary constituents and toxicants have been given in Table 17 and discussed briefly in Sect. 7.5.1. In this section the focus is on enzyme induction and its role in inhibition of neoplasia.

9.4.1 Enzyme Induction by Dietary Constituents

Many xenobiotics are capable of inducing microsomal enzymes. These inducers are of at least two types, exemplified by phenobartital (P-450) and 3-methylcholanthrene (P-448) (Conney 1967). The microsomal mixed-function oxidase system is a complex biochemical entity that metabolizes a wide variety of xenobiotics including chemical carcinogens. Three components of the system have been identified: cytochrome P-450, cytochrome P-450 reductase, and a lipid. Two distinctive cytochromes have been identified in liver microsomes: naturally occurring P-450 and a closely related species, P-448 (also called P_1-450). Considerable work has been done on one of the components of the cytochrome P-450 system, aryl hydrocarbon hydroxylase (AHH) activity, which is induced by many chemicals and dietary factors. A basal level of AHH activity is present in the liver but not in the small intestine or lungs. AHH activity and also glutathione sulfur-transferase activity was shown to be induced in these tissues by natural ingredient diets such as Purina Rat Chow (Wattenberg 1982). Later investigations revealed that most of the inducing activity was associated with the vegetable component, which consisted of alfalfa meal. Naturally occurring inducers were found in cruciferous plants such as brussels sprouts, cabbages, and cauliflower and identified as indole derivatives. Flavones naturally occurring in citrus fruits, safrole, iso-safrole, and β-ionone have also been found to be enzyme inducers (Wattenberg 1975).

9.4.2 Enzyme Induction by Feed Additives

Preservatives, butylated hydroxyanisole and ethoxyquin, have been shown to be good enzyme inducers and consequently reduce the carcinogenic effect of chemicals such as benzo(a)pyrene, 7,12-dimethylbenzanthracene, and dimethylnitrosaniline (Wattenberg 1979) (Table 16). However, in these experiments, much higher levels of preservatives (5-10 mg/g) were used than is normally present in animal feed (8-20 μg/g).

9.4.3 Enzyme Induction by Contaminants in Animal Feed

Data on enzyme induction by pesticide residues in animal feed are rather scarce. Chlordane, DDT, hexachlorocyclohexane, dieldrin, aldrin, heptachlorepoxide, and pyrethrums have been reported to be inducers of enzyme activity (Conney 1967) and, therefore, if present in animal diets, have the potential to alter the outcome of long-term toxicity experiments by inducing enzymes that may alter the metabolism of the chemical under study.

9.4.4 Inhibition of Neoplasia

Food contains a large number of inhibitors of carcinogenesis such as phenols, indoles, aromatic isothiocyanates, methylated flavones, coumarins, plant sterols, selenium salts, protease ihibitors, ascorbic acid, tocophorols, retinol, and carotenes. At present, the mechanism of inhibition of carcinogenesis by these factors is poorly understood. An attempt has been made to classify these inhibitors into three different classes according to the time in the carcinogenic process that they are effective. The compounds that prevent the formation of ultimate carcinogenesis from precursors belong to the first group (e.g., α-tocopherol, ascorbic acid). In the second group are compounds that inhibit carcinogenesis by preventing carcinogenic agents from reaching or reacting with critical target sites in the tissues (so-called blocking agents). The third category of inhibitors acts subsequent to exposures to carcinogens (so-called "suppressive agents"). Of these three categories, the second category or the "blocking agents" most often act as inducers of enzymes (Wattenberg 1983).

9.4.5 Monitoring of Induced Enzyme Activity

The most frequently monitored induced enzyme activities are aryl hydrocarbon hydroxylase (Wiebel et al. 1974) and glutathione S-transferase activity (Sparnins et al. 1982). UDP-glucuronyltransferase, epoxide hydratase (Wattenberg 1980), aminopyrene demethylase, p-nitroanisole demethylase, acid phosphatase, and glucose phosphatase (Newberne 1982) have also been monitored.

9.4.6 Enzyme Induction and Genetic Differences

Induction of cytochrome P_1-450 (also called P-448) by polycyclic aromatic compounds has been manifested in the B6 mouse (the inbred C57BL/6 strain) and in other responsive inbred mouse strains, but induction of this form of cytochrome by polycyclic aromatic hydrocarbons is absent in the liver and markedly decreased in all other tissues examined in the D2 (the inbred DBA/2 mouse strain) and other nonresponsive inbred mouse strains. This responsiveness to aromatic hydrocarbons has been termed the Ah locus: the allele Ah^b denotes the B6, and Ah^d the D2 inbred mouse (Nebert et al. 1977).

It has been shown that when mice are given massive intraperitoneal doses of benzo(a)pyrene, the survival time of the three responsive inbred strains - B6, C3H/HeN, and BALB/cAmN - is significantly shorter than that of the two nonresponsive inbred strains, D2 and AHR/N. In contrast, when benzo(a)pyrene is administered orally in smaller daily doses, the nonresponsive mouse survives less than 4 weeks, whereas no significant earlier death rate is seen in genetically responsive mice, even after ingesting benzo(a)pyrene for 6 months. Similar differences have been observed when benzo(a)pyrene was substituted by arochlor 1254, lindane, or hexachlorobenzene. Kepone has also been shown to induce AHH activity in B6 but not in D2 mice (Nebert et al. 1977).

10. SPECIAL TOPICS

Phytates, arsenic, and selenium are treated separately in this section due to the special concern about them in recent years.

10.1 Phytates

Oberleas (1973) provides a comprehensive discussion of phytates as a naturally occurring toxicant in food. Of all the ingredients used in animal feeds, probably soybean meal and wheat are the richest sources of phytate. The phytic acid from plant sources has been identified to be myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate). Consideration of phytates in animal nutrition is important because they can interfere with the absorption of metal ions from the gut. At pH 7.4 phytates form complexes with metals in the following decreasing order: $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+}$. An important factor in the precipitation of metal phytates is the synergistic effect produced when two or more metal ions are present simultaneously; by acting together they increase the quantity of metal phytate precipitated. This phenomenon has been demonstrated for Zn and Ca and for Cu and Ca. Oberleas states that when diets contain plant materials (e.g., soybean) rather than casein as a major source of protein, it is advisable to increase the amount of Zn intake. For this reason the AIN-76™ purified rat and mouse diet uses 2.5 times the level of Zn recommended by NAS (see Tables 1 and 2, respectively).

Calcium absorption in the gut is influenced by phytate, vitamin D, and other dietary factors. Diets that have enough Ca, P, and vitamin D and calcium: inorganic phosphorus ratios between 1:1 and 2:1, respectively, are not likely to be rachitogenic even though much calcium may be bound to the phytates.

10.2 Arsenic

The role of arsenic (As) in the animal diet is controversial (Shendriker 1982, Huff 1982, Frost 1982). Although there is inadequate evidence for the carcinogenicity of arsenic compounds in animals, there is sufficient evidence that inorganic arsenic compounds are skin and lung carcinogens in humans (IARC 1980). Recently Frost (1982) has focused on the noncarcinogenicity of As and on its anticarcinogenicity. High As intake has led to reduced spontaneous lung cancer in mice (Kanisawa and Schroeder 1967, 1969), and epidemiological data indicate a correlation between low As intake and high lung cancer rate (Schrauzer 1978). Nielsen et al. (1975) have shown that the offspring of Sprague-Dawley dams placed on a purified diet containing approximately 30 ppb As, and maintained in a plastic isolator environment, displayed a rough coat and a significantly slower rate of growth than controls receiving a supplement of 4.5 ppm As as sodium arsenate (4 ppm) and arsenite (0.5 ppm). At 12-15 weeks the deficient males exhibited significantly low hematocrits and enlarged and blackened spleens containing 50% more iron on a per gram basis. The beneficial effects of As on the growth, health, and feed efficiency of pigs and poultry have been well established (Frost 1967).

4-Nitrophenylarsenic acid and 3-nitro-4-hydroxyphenylarsonic acid have been found to be effective growth stimulants in pigs and poultry, and phenylarsenoxides are more potent than arsenic acids as coccidiostats. Arsenic can act as an antagonist to selenium (Se) and has been used successfully to alleviate Se poisoning in animals (Underwood 1977). Whether As should be treated as a micronutrient like Se in animal nutrition is still an open question. Unfortunately, no information documenting safe levels for long-term feeding studies is available.

10.3 Selenium

The role of selenium (Se) as an essential micronutrient in animal feeds has been well established. It has been shown to be a constituent of glutathione peroxidase, an enzyme involved in the disposal of peroxides in body tissues. Selenium and vitamin E supplement each other in most animal species studied except in rabbits where the relationship does not hold (NAS 1977).

Superiority of natural Se over selenite Se in increasing the Se levels of muscle and liver has been demonstrated in laying hens (Latshaw 1975). Biological availability of Se was shown to be only 54-58% as great from tuna as from selenite for induction of glutathione peroxidase in liver and in red blood cells of rats (Douglass et al. 1981). The Se in most of the feed stock of plant origin was highly available, ranging from 60-90%, but was less than 25% available when the feed stock was of animal origin. This assay was based on the prevention of exudative diathesis induced in chicks on a Se-deficient diet (Cantor et al. 1975).

Although Se is an essential micronutrient, an overdose causes restriction in food intake in rats and dogs together with anemia and severe pathological changes in the liver (Moxon and Rhian 1964). Biliary Se excretion is increased when subacute doses of As are administered. Arsenic has been used as an antidote to Se poisoning, and mercury, copper, and cadmium are also known to interact with Se and offer protection against Se poisoning (Underwood 1977). Contrary to earlier experiments, Se has been demonstrated to have an anticarcinogenic effect (Weisberger and Suhrland 1956). Selenium (sodium selenite, ~0.5 mg/week) is undergoing clinical trial for the treatment of endemic cardiomyopathy in China (Sadler 1982).

Selenium requirements in animal feeds are usually met by the selenium naturally occurring in feed stock. However, when naturally insufficient, it may be necessary to add Se to the animal feed. At present selenium is recognized as a carcinogen, and its incorporation into commercially prepared laboratory animal feed is subject to the Delaney anticancer clause in the Federal Food, Drug, and Cosmetic Act; but in view of the fact that Se is an essential micronutrient in laboratory animal diets, the Food and Drug Administration has decided not to enforce this regulation in this particular case. The legal position of the Food and Drug Administration is explained by Ballitch (see Appendix C).

11. ANALYSIS OF PUBLIC COMMENTS ON THE PROPOSED EPA GUIDELINES FOR DIETARY REQUIREMENTS AND CONTAMINANT ANALYSIS

11.1 Criticisms of Proposed Guidelines and Related Discussion

Several commenters agreed with the EPA that a standardized diet should be required and should meet minimal requirements because dietary constituents can influence the incidence of disease, including neoplasms, and certain dietary contaminants may be linked to synergistic, additive or antagonistic effects in animal studies. Many commenters, however, disagreed with the EPA approach on several grounds:
(1) feasibility, (2) practicality, (3) scientific justification,
(4) lack of harmony with FDA, USDA, etc., (5) cost effectiveness,
(6) lack of incentive to continue research on diet development, and
(7) failure to promote standardization of the diet. The changes suggested in the EPA approach include (1) deletion of Appendix A and B from the proposed guidelines and (2) adoption of a different set of criteria for diet standards.

The focus of EPA's concern in these proposed guidelines is long-term toxicity testing of chemicals using laboratory animals. The fact that dietary constituents and contaminants can affect the outcome of the tests is well recognized. The customary use of a set of control animals for the purpose of comparing with the exposed animals is not an adequate safeguard since dietary variables can have significant interactions with experimental variables or parameters (Greenman et al. 1980). These considerations justify the EPA approach to set the guidelines for the dietary requirements and contaminant analysis. However, most laboratories procure their animal feed from commercial sources. A major share of the burden of complying with the EPA regulations will thus fall on the laboratory animal feed manufacturers. As testified by several commenters, the industry has established a good record of adequate service to the needs of the scientific community. The commenters feel that the EPA regulations should be flexible and permit the investigator and the feed manufacturer a greater latitude in the choice of feed ingredients, use of preservatives, and analytical requirements. As an example of the difficulty that feed manufacturers have in ensuring the use of proper ingredients, consider alfalfa, which used to be oven-dried but now is mostly field-dried for economic reasons with consequent reduction in quality of the resulting feed.

It is customary to analyze the vitamins and minerals in the premixes and to analyze one or two components in the finished product to ensure proper distribution. Vitamins that are partially destroyed during the manufacturing process, such as vitamin C, have to be assayed in the finished product. Some of the analysis may be costly, time-consuming and unnecessary if carried out routinely; for instance estrogenic activity is usually not assayed unless it is relevant to the particular experiment. The analytical burden on the industry should be kept at a minimum without compromising the objectives of the EPA Good Laboratory Practice. It should be noted that as is evident from the data presented in Table 1, the animal feed industry is already meeting many of the standards set by the EPA.

11.2 Analysis of Specific Comments

11.2.1 Shelf Life Restrictions

Numerous commenters argued that the restriction on shelf life (90 days) is unwarranted. Consideration should be given for the use of preservatives such as ethoxyquin and BHA, except where these compounds are known to interact with the chemical under investigation. In fact, as shown in Table 8, NIH diet formulations do use preservatives.

If preservatives are allowed, the shelf life of most laboratory feeds is accepted to be about 6 months except in case of guinea pig feed where the shelf life is usually 3 months because of rapid deterioration of vitamin C. However, if supplemented with vitamin C, shelf life of guinea pig feed can also be extended to 6 months. These shelf life data are valid only when the feed is kept at 70°F or lower and a relative humidity of 50% under clean storage conditions (personal communication from D.C. Shelton of Ralston Purina 1982).

11.2.2 Feed Meal

Justification of the requirement that the feed in meal form should be manufactured by regrinding pellets has been questioned. Pelletized feed for long-term studies has been suggested. Joseph Knapka of NIH has retracted his statement (Knapka 1979) that volatile nitrosamine levels in animal feed are substantially lowered during the pelletizing process (personal communication from J. Knapka 1982); therefore, there apparently is little justification for preparing the feed in meal form by regrinding the pellets, although it may be preferable to do so to minimize biological contaminants and to subject the feed to the same heat treatment as the pellets. Whether autoclaving reduces nitrosamine concentrations in the feed, as suggested by one commenter, needs to be documented.

11.2.3 Calcium, Phosphorus, Iron, and Cobalt

The EPA proposed minimum for calcium (Ca) and phosphorus (P) for rodent diets are 1.15% and 0.9%, respectively. Calcium and phosphorus in some commercial rodent diets range from 0.9% to 1.1% and from 0.6% to 0.85%, respectively (see Table 1). One commenter stated that the EPA minimum levels for Ca and P are rather high. According to NAS (1978) the Ca:P ratio in the diet is more important than their absolute percentage in the feed and should be greater than one. Consideration should be given to lowering the proposed minimum levels of Ca and P in the diet and also for including a specified minimum for the Ca:P ratio.

The EPA proposed minimum for iron in rodent diets is 345 ppm. Levels of iron in some commercial rodent diets range from 180 ppm to 298.7 ppm (see Table 1). The NAS recommendation for iron in rodent diets ranges from 25 ppm to 140 ppm (see Table 2). As has been pointed out by one commenter, the minimum level of iron in the proposed EPA guidelines seems rather high, and consideration should be given to lowering the level of iron.

One commenter stated that cobalt is not a requirement under normal animal husbandry. This agrees with the NAS recommendation for the rat diet. However, cobalt is a requirement for the golden hamster; the amount required in the diet has been estimated to be 1.1 ppm (see Table 2) (NAS 1978). It is required for the synthesis of vitamin B_{12}, and all commercial rodent feed formulations include cobalt, 0.38 ppm to 0.96 ppm (Table 1), to ensure proper nutrition.

11.2.4 Vitamins

The minimum level of vitamin K proposed in the EPA guidelines is 3 ppm. One commenter has pointed out that the vitamin K level of 3 ppm is totally inadequate for normal blood clotting in certain widely used strains of rodents. The most satisfactory level appears to be 15 ppm according to this commenter. Rodents meet a large part of the dietary need for vitamin K through intestinal synthesis and coprophagy (NAS 1978). Purina rodent chow #5002, guinea pig chow #5026, and rabbit chow #5322 do not include vitamin K (Table 1); however, the Agway R-M-H 3000 diet does include 0.97 ppm of vitamin K (Table 1). These facts favor the retention of the proposed level; however, the requirement for higher levels in certain strains of rodents should be further investigated. One commenter questioned the absence of vitamin C in the proposed guidelines. Vitamin C is required in guinea pig diet formulations (NAS 1978). Other animals do not require a dietary source of vitamin C, and commercial diet formulations for the mouse, rat, dog, hamster, and rabbit do not include vitamin C (Table 1). Since the guinea pig is a rodent the EPA guidelines should include vitamin C if they are intended for all rodents.

As pointed out by one commenter, alternative sources of thiamine (thiamine hydrochloride) and niacin (niacinamide) should be considered for inclusion in the proposed EPA guidelines to allow flexibility in diet formulations.

A question was raised on whether EPA should allow the use of vitamin D-activated vegetable sterol along with vitamin D-activated animal sterol. Vitamin D-activated animal sterol is a source of vitamin D_3, and vitamin D-activated vegetable sterol is a source of vitamin D_2 (AAFCO 1982). Although no information was available for rodents, vitamin D_3 is preferred over D_2 for poultry diets (Merck Index 1976). The commercial diet formulations listed in Table 1 use vitamin D-activated animal sterol. No data are readily available to suggest that the use of vitamin D-activated vegetable sterol be permitted.

11.2.5 Choline and Inositol

One commenter observed that the minimum level of choline proposed in the EPA guidelines (USEPA 1979) is too high (1900 ppm). The guaranteed minimum level of choline in rodent diets varies from 1542 ppm to 2000 ppm in commercial diets (Table 1). The NAS recommendation for choline in the diet of different animals ranges from a low of 600 ppm (mouse) to a high of 2000 ppm (golden hamster) (Table 2). No reason is readily apparent for changing the proposed EPA value unless it is desired to provide values for individual species.

One commenter questioned the absence of inositol in the EPA guidelines. Inositol is not normally required in the diet of laboratory animals. None of the commercial diet formulations include inositol (see Table 1). However, the NAS recommended diet for the golden hamster does require 100 ppm of inositol (NAS 1978) (see Table 2).

11.2.6 Alternative Sources of Minerals

One commenter pointed out that the permitted sources of minerals include manganese oxide and cobalt carbonate but do not include manganese sulfate nor cobalt sulfate. Since the two latter compounds are included in the GRAS list (see Table 7), their use in the EPA guidelines should be considered to allow flexibility in the diet formulation.

11.2.7 Dicalcium Phosphate

One commenter expressed concern about the potential effects of fluorine usually associated with dicalcium phosphate, which is specified as a source of minerals in the proposed guidelines. Specifications for dicalcium phosphate include a maximum permissible level of fluorine of no more than 1 part to 100 parts of phosphorus (AAFCO 1982). Most commercial diet formulations do not state the amount of fluoride present; the Agway R-M-H 3000 diet is an exception and contains an average of 35 ppm of fluoride (see Table 1). Consideration should be given for specifying an acceptable level of fluoride in the diet.

11.2.8 Fat

The minimum percentage of fat allowed in the proposed EPA guidelines is 4.3%. One commenter remarked that in the case of adult, nonbreeding rodents, the use of a lower percentage (2-3%) of fat in the diet avoids obesity and perhaps shortening of life span. Commercial rodent diets include 4-5% fat (see Table 1). The NAS recommendation for the rodent diet also recommends inclusion of 5% fat (see Table 2).

11.2.9 Ash

One commenter stated that the minimum ash content of 8% in the EPA guidelines is rather excessive, particularly for adult rodents. The maximum ash content of some commercial rodent feeds is guaranteed to be 6% (Agway Inc. n.d.) or 7% (Ralston Purina Company 1980). The specified ash content in the EPA guidelines possibly should be revised accordingly.

11.2.10 Air Dry

One commenter asked for a definition of "air dry" because the EPA guidelines specify that the nutrient content of both ingredients and the finished product must be expressed as a nutrient content percentage by weight on an air-dry basis. Since normal storage conditions of animal feed specify a temperature of 70°F and a relative humidity of 50%, "air dry" feed may be defined as the feed dried to a constant weight under these conditions. Alternatives are to report the results on a dry weight basis or 90% dry matter basis as reported in NAS (1974).

11.2.11 Units of Measurement

For the sake of uniformity, consideration should be given to using ppm instead of mg/kg. Vitamins A and D are usually expressed in I.U./g. The use of units such as Mcg/lb should be avoided, as indicated by one commenter.

LITERATURE CITED

AAFCO. 1982. Association of American Feed Control Officials Inc. Official publication. Department of Agriculture, Charleston, WV.

Agway Inc. (n.d.). Technical data sheet-certified rat-mouse-hamster R-M-H 3000 diet and autoclavable R-M-H 3500 diet (A-4540A5-80), certified canine diet respond 2000 (A-4608 5-80), certified guinea pig formula (A-4544 5-80), and certified rabbit diet (A4546A 5-80). Box 4933, Syracuse, NY 13221.

AIN. 1977. Report of the American Institute of Nutrition. Ad Hoc committee on standards for nutritional studies. J Nutr 107(7):1340-1348.

AOAC. 1980. Association of Official Analytical Chemists. Official methods of analysis. Thirteenth edition. Published by the Association, Washington, D.C.

APHA. 1965. American Public Health Association. Standard methods for the examination of water and wastewater, 12th ed. New York, pp. 187-193. Reviewed in Greenman et al. 1980.

Becking GE. 1976. Hepatic drug metabolism in iron-, magnesium-, and potassium-deficient rats. Fed Proc 35:2480-2485.

Beljaars PR, Schumans JCHM, Koken PJ. 1975. Quantitative fluorodemitometric determination and survey of aflatoxins in nutmeg. J Assoc Off Anal Chem 58:263-271. Reviewed in Greenman et al. 1980.

Bettegar WJ, Reeves PG, Moscatelli EA. 1979. Interaction of zinc and essential fatty acids in the rat. J Nutr 109:480-488.

Bowman MC. 1973. Modifications of AOAC method, 11th ed., 1970. NCTR Chem Div Rep, July 20, pp. 771-774. Reviewed in Greenman et al. 1980.

Bowman MC, Beroza M. 1970. GLC retention times of pesticides and metabolites containing phosphorus and sulfur on four thermally stable columns. J Assoc Off Anal Chem 53:499-508. Reviewed in Greenman et al. 1980.

Bowman MC, Beroza M, Hill KR. 1971. Chromatograms of food for multicomponent residue determination of pesticides containing phosphorus and/or sulfur by GLC with flame photometric detection. J Assoc Off Anal Chem 54:346-358. Reviewed in Greenman et al. 1980.

Bowman MC, King JM. 1973. Modification of AOAC method, 11th ed., 1970. NCTR Chem Div Rep, Sept. 27, pp. 767-769. Reviewed in Greenman et al. 1980.

Boyd EM. 1969. Dietary protein and pesticide toxicity in male weanling rats. Bull WHO 49:801-805.

Brinkman GL, Miller RF. 1961. Influence of cage type and dietary zinc oxide upon molybdenum toxicity. Science 134:1531-1532.

Brody SS, Chaney JE. 1966. Flame photometric detector: The application of a specific detector for phosphorus and for sulfur compounds sensitive to subnanogram quantities. J Gas Chromatogr 4:42-46. Reviewed in Greenman et al. 1980.

Bryon WR, Bierbower GW, Brouwer JB, Hansen WH. 1967. Pathologic changes in rats and dogs from two-year feeding of sodium arsenite or sodium arsenate. Toxicol Appl Pharmacol 10:132-147. Reviewed in Greenman et al. 1980.

Burchfield H, Johnson D, eds. 1965. Extraction and cleanup of monionic pesticides. In: Guide to the analysis of pesticide residues, Vol. 1, Sec. 11 A.7. Washington, DC: Department of Health, Education and Welfare. Reviewed in Greenman et al. 1980.

Burkitt DP. 1971. Epidemiology of cancer of the colon and rectum. Cancer 28:3-13.

Burkitt DP. 1976. A deficiency of dietary fiber may be one cause of certain colonia and venous disorders. Am J Dig Dis 21(2):104-108. Biological Abstract 44656, 1976 (Oct. 15).

Cantor AH, Scott ML, Noguchi T. 1975. Biological availability of selenium in feedstuffs and selenium compounds for prevention of exudative diathesis in chicks. J Nutr 105:96-105.

Carr CJ. 1982. Food and drug interactions. In: Ann Rev Pharm Toxic 22:19-29, eds. AK Cho, R Okun and R George. Ann Rev Inc, California.

Carroll KK, Khor HT. 1970. Effects of dietary fat and dose level of 7,12-dimethylbenz(a)anthracene on mammary tumor incidence in rats. Cancer Res 30:2260-2264.

Cecil HC, Harris SJ, Bitman J. 1974. Effects of nonpersistent pesticides on liver weight, lipids and vitamin A of rats and quail. Bull Environ Contam Toxicol 11:496-499. Reviewed in Greenman et al. 1980.

Chah JC. 1982. Personal communication to B.C. Pal. Zeigler Bros. P.O. Box 95, Gardners, PA 17324.

Clarke HE, Coates ME, Eva JK, et al. 1977. Dietary standards of laboratory animals: report of the laboratory animals centre diets advisory committee. Lab Animals 11:1-28.

Cone MV, Nettesheim P. 1973. Effects of vitamin A on 3-methylcholanthrene-induced squamous nutaplasias and early tumors in the respiratory tract of rats. J Natl Cancer Inst 50:1599-1606.

Conney AH. 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol Rev 19:317-366.

Den Tonkelaar EM, Van Esch GJ. 1974. No-effect levels of organochlorine pesticides based on induction of microsomal liver enzymes in short-term toxicity experiments. Toxicology 2:371-380. Reviewed in Greenman et al. 1980.

Desi I, Dura G, Gonczi L, Kneffel L, Strohmayer A, Szabo Z. 1976. Toxicity of malathion to mammals, aquatic organisms and tissue culture cells. Arch Environ Contam Toxicol 3:410-425. Reviewed in Greenman et al. 1980.

Douglass JS, Morris VC, Soares JH Jr, Levander OA. 1981. Nutritional availability to rats of selenium in tuna, beef kidney, and wheat. J Nutr 111:2180-2187.

Dutchland Laboratories Inc. 1982. Commercial literature. Swampbridge Road, Box 139-A, Denver, PA 17517.

Edwards GS, Fox JG. 1979. Significance of nitrosamines in animal diets. Science 203:6.

Edwards GS, Fox JG, Policastro P, Groff U, Wolf MH, Fine DH. 1979. Volatile nitrosamine contamination of laboratory animal diets. Cancer Res 39:1857-1858.

Epstein SS. 1976. Carcinogenicity of heptachlor and chlordane. Sci Total Environ 6:103-154. Reviewed in Greenman et al. 1980.

Fan TY, Tannembaum SR. 1973. Natural inhibitors of nitrosation reactions: the concept of available nitrite. J Food Sci 38:1067-1969.

Farkas CS. 1978. Importance of interactions between nutrients and environmental contaminants as a factor in experimental design in toxicological research, with emphasis on selenium and ascorbic acid. Sci Total Environ 9(2):148-160.

Farm Chemicals Handbook. 1981. Willoughby, OH: Meister Publishing Co.

Festing MFW. 1979. Inbred strains in biomedical research. New York: Oxford University.

Fodor G, Kemeny GL. 1965. On the hepato-protective effect of selenium in carbon tetrachloride poisoning in albino rats. Experentia 21:666-667.

Ford J. 1982. Personal communication to B.C.Pal. Ralston Purina Company. Richmond, IN 47374.

Fowler BA. 1972. The morphologic effects of dieldrin and methyl mercuric chloride on pars recta segments of rat kidney proximal tubules. Am J Pathol 69:163-178. Reviewed in Greenman et al. 1980.

Fowler BA, Jones HS, Brown HW, Haseman JK. 1975. The morphologic effects of chronic cadmium administration on the renal vasculature of rats given low and normal calcium diets. Toxicol Appl Pharmacol 34:233-252. Reviewed in Greenman et al. 1980.

Foster HL, Small JD, Fox JG, eds. 1981. The mouse in biomedical research. New York: Academic.

Frost D. 1982. Selenium/arsenic vs. cancer. Chem Eng News, April 12, p. 4.

Frost DV. 1967. Arsenicals in biology - retrospect and prospect. Fed Proc, Fed Am Soc Exp Biol 26:194

Frost DV. 1977. The arsenic problems. In: Inorganic and nutritional aspects of cancer, ed. G.N. Schrauzer, pp. 259-279. Plenum Press, London.

Gillett JW. 1968. "No effect" level of DDT in induction of microsomal epoxidation. J Agric Food Chem 16:295-297. Reviewed in Greenman et al. 1980.

Gillett JW, Chan IM. 1968. Cyclodiene insecticides as inducers, substrates, and inhibitors of microsomal epoxidation. J Agric Food Chem 16:590-593. Reviewed in Greenman et al. 1980.

Goldschmidt S, Vars HM, Raudin IS. 1939. Influence of the foodstuffs upon the susceptibility of the liver to injury by chloroform and the probable mechanism of their action. J Clin Invest 18:277-289.

Greenman DL, Oller WL, Littlefield NA, Nelson CJ. 1980. Commercial laboratory animal diets: toxicant and nutrient variability. J Toxicol Environ Health 6:235-246.

Haber L, Wissler RW. 1962. Effect of vitamin E on carcinogenicity of methylcholanthrene. Proc Soc Exp Biol Med 111:774-775.

Hamm TE Jr., Raynor T, Caviston T. 1982. Unsuitability of the AIN 76A diet for male F-344 and CD rats and improvement by substituting starch for sucrose. Lab Animal Sci 32(4):414.

Harr JR, Claeys RR, Benedict N. 1970. Dieldrin toxicosis in rats: Long-term study of brain and vascular effects. Am J Vet Res 31:1853-1862. Reviewed in Greenman et al. 1980.

Harr J, Exon J, Weswig P, Whanger P. 1973. Relationship of dietary selenium concentration, chemical cancer induction, and tissue concentration of selenium in rats. Clin Toxicol 6:487-495. Reviewed in Greenman et al. 1980.

Hazelton L, Holland E. 1953. Toxicity of malathion. Summary of mammalian investigations. AMA Arch Ind Hyg Occup Med 8:399-405. Reviewed in Greenman et al. 1980.

Heckman M. 1968. Collaborative study of minerals in feeds by atomic adsorption spectrophotometry. J Assoc Off Anal Chem 51:776-779. Reviewed in Greenman et al. 1980.

Hedrick HJ. 1981. Genetic monitoring. In: The mouse in biomedical research, Vol. 1.

Hoffman I, Westerby RJ, Hidiroglou M. 1968. Precise fluorometric microdetermination of selenium in agricultural materials. J Assoc Off Anal Chem 51:1039-1042. Reviewed in Greenman et al. 1980.

Hoffman-LaRoche Inc. (n.d.) Stability of ascorbic acid in feeds. Nutley, NJ.

Holtzman NA. 1982. The promise and perils of genetic prophecy. Chem Eng News, Aug. 2. p. 23.

Horwitz W., ed. 1970a. Animal feed. In: Official methods of analysis of the association of official analytical chemists, 11th ed., Sec. 7.047, 7.072, 7.087. Washington, DC: AOAC. Reviewed in Greenman et al. 1980.

Horwitz W., ed. 1970b. Metals and other elements as residues in foods. In: Official methods of analysis of the association of official analytical chemists, 11th ed., Sec. 25.008, 25.026, 25.017, 25.041-25.043. Washington, DC: AOAC. Reviewed in Greenman et al. 1980.

Horwitz W., ed. 1970c. Natural poisons. In: Official methods of analysis of the association of official analytical chemists, 11th ed., Sec. 26.015. Washington, DC: AOAC. Reviewed in Greenman et al. 1980.

Horwitz W., ed. 1970d. Pesticide residues. In: Official methods of analysis of the association of official analytical chemists, 11th ed., Sec. 29.011, 29.067. Washington, DC: AOAC. Reviewed in Greenman et al. 1980.

Hubermont G, Buchet J, Roels H, Lauwerys R. 1976. Effect of short-term administration of lead to pregnant rats. Toxicology 5:379-384. Reviewed in Greenman et al. 1980.

Huff JE. 1982. Trace elements and cancer. Chem Eng News, Jan 18, p. 97.

Hundley HK, Underwood JC. 1970. Determinations of total arsenic in total diet samples. J Assoc Off Anal Chem 53:1176-1178. Reviewed in Greenman et al. 1980.

IARC. 1980. International agency for research on cancer. Arsenic. Monograph 23:114.

J Assoc Off Anal Chem 1971. Flameless atomic absorption method official first action. Sec. 25.A02-25.A03. 54:466-467.

J Assoc Off Anal Chem. 1973a. Atomic absorption spectrophotometric method official first action. Sec 25.C07-25.C11. 56:480-481. Reviewed in Greenman et al. 1980.

J Assoc Off Anal Chem. 1973b. Lead in evaporated milk: Atomic absorption spectrophotometer method official first action. Sec. 25.C15-25.C17. 56:481-482. Reviewed in Greenman et al. 1980.

Kahl R, Klaus E. 1979. Effect of dietary antioxidants on epoxide hydratase and on DNA modifications by benzo(a)pyrene. Hoppe Seylers Z Physiol Chem 360(3):294.

Kanisawa M, Schroeder HA. 1967. Life term studies on the effects of arsenic, germanium, tin, and vanadium on spontaneous tumors in mice. Cancer Res 27:1192.

Kanisawa M, Schroeder HA. 1969. Life term studies on the effect of trace elements on spontaneous tumors in mice and rats. Cancer Res 29:892.

Kinoshita FK, Frawley JP, DuBois KP. 1966. Quantitative measurement of induction of hepatic microsomal enzymes by various dietary levels of DDT and toxaphene in rats. Toxicol Appl Pharmacol 9:505-513. Reviewed in Greenman et al. 1980.

Knapka JJ. 1979. Laboratory animal feed. Science 204:1367.

Knapka JJ. 1982. Personal communication to B.C.Pal. National Institutes of Health. Bethesda, MD 20205.

Knapka JJ, Smith KP, Judge FJ. 1974. Effect of open and closed formula rations on the performance of three strains of laboratory mice. Lab Animal Sci 24(3):480-487.

Kolata G. 1982. Value of low sodium diets questioned. Science 216:38-39.

Koller LD, Kovacic S. 1974. Decreased antibody formation in mice exposed to lead. Nature (Lond.) 250:148-150. Reviewed in Greenman et al. 1980.

Latshaw JD. 1975. Natural and selenite selenium in the hen and egg. J Nutr 105:32-37.

Laug EP, Nelson AA, Fitzhugh OG, Kunze FM. 1950. Liver cell alteration and DDT storage in the fat of the rat induced by dietary levels of 1 to 50 ppm DDT. J Pharmacol Exp Ther 98:268-273. Reviewed in Greenman et al. 1980.

Lee S, Chu F. 1981. Radioimmunoassay of T-2 toxin in corn and wheat. J Assoc Off Anal Chem 64(1):156-161.

Lieber CS, DeCarli LM. 1966. Agents for the prevention of fatty liver produced by prolonged alcohol intake. Gastroenterology 50(3):316-22.

Lindenfelser LA, Lillchoj EB, Sansing GA. 1976. Toxicity to white mice of corn naturally contaminated with aflatoxin. J Agric Food Chem 24:198-200. Reviewed in Greenman et al. 1980.

Merck Index. 1976. Published by Merck & Co., Rahway, NJ.

Marshall WJ, McLean AEM. 1969. A requirement for dietary lipids for induction of cytochrome P-450 by phenobarbitone in rat liver microsome fraction. Biochem J 122:569-573.

McLean AEM, Verschuuren HG. 1969. Effects of diet and microsomal enzyme induction on the toxicity of dimethylnitrosamine. Br J Expl Pathol 50:22-25.

McMahon B, Sawyer L., eds. 1975a. Extraction and cleanup. In: Pesticide analytical manual, Vol. 1, Sec. 212. Washington, DC: Food and Drug Administration. Reviewed in Greenman et al. 1980.

McMahon B, Sawyer L., eds. 1975b. Gas-liquid chromatography. In: Pesticide analytical manual, Vol. 1, Sec. 301. Washington, DC: Food and Drug Administration. Reviewed in Greenman et al. 1980.

Meneely GR. 1973. Toxic effects of dietary sodium chloride and the protective effects of potassium. In: Toxicants Occurring Naturally in Foods. National Academy of Sciences. Washington, DC.

Miller JA. 1973. Naturally occurring substances that can produce tumors. In: Toxicants occurring naturally in foods. Natl Acad Sci, Washington, DC. pp. 508-549.

Moxon AL, Rhian M. 1964. Selenium poisoning. Physiol Rev 23:305.

NAS. 1974. National Academy of Sciences. Nutrient requirements of dogs. Number 8.

NAS. 1977. National Academy of Sciences. Nutrient requirements of rabbits.

NAS. 1978. National Academy of Sciences. Nutrient requirements of laboratory animals.

NAS. 1978a. National Academy of Sciences. Control of diets in laboratory animal experimentation. Washington, DC.

NIH. 1980. National Institutes of Health. Animal diets and diet quality assurance at the National Institutes of Health, Knapka JJ. Personal communication to B.C. Pal.

Nebert DW, Levitt RC, Orlando MM, Felton JS. 1977. Effects of environmental chemicals on the genetic regulation of microsomal enzyme systems. Clin Pharmacol and Therapeutics 22(5) part 2:640-658.

The New York Times. 1982. Wheat-disease rise worrying midwest. Vomitoxin and zearalenone (wheat scab). August 2.

Newberne PM. 1975. Influence of pharmacological experiments of chemicals and other factors in diets of laboratory animals. Fed Proc 34:209.

Newberne PM. 1982. Assessment of the hepatocarcinogenic potential of chemicals: response of the liver. In: Toxicology of the liver. Plaa G, Hewitt WR, eds. New York: Raven.

Newberne PM, Bieri LG, Briggs GM, Nesheim MC. 1978. Control of diets in laboratory animal experimentation. ILAR News, XXI(2), 1978. National Academy of Sciences, Washington, DC.

Newberne PM, Butler WH. 1969. Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: A review. Cancer Res 29:236-250. Reviewed in Greenman et al. 1980.

Newberne PM, McConnell RG. 1981. Dietary nutrients and contaminants in laboratory animal experimentation. J Environ Pathol Toxicol 4:105-122.

Newberne PM, Rogers AE. 1976. Nutritional modulation of carcinogenesis. In: Fundamentals in cancer prevention, P.N. Magee et al., eds. University of Tokyo Press, Tokyo/University Park Press, Baltimore. pp. 15-40.

Newberne PM, Rogers AE, Wogan GN. 1968. Hepatorenal lesions in rats fed a low lipotrope diet and exposed to aflatoxin. J Nutr 94:331-343.

Nguyen HT. 1982. Nephrocalcinosis induced by AIN-76 semi-purified diets in rats: a nutritional and ultrastructural study. Lab Animal Sci 32(4):415.

Nielson FH, Givand SH, Myron DR. 1975. Evidence of a possible requirement for arsenic in the rat. Fed Proc, Fed Am Soc Exp Biol 39:923 (abstr).

NRC. 1976. National Research Council. Long-term holding of laboratory rodents. ILAR News 19(4):L1-L25.

Oberleas D. 1973. Phytates. In: Toxicants occurring naturally in foods. Nat Acad Sci, Washington, DC. pp. 363-371.

Olson K, Boush GM. 1975. Decreased learning capacity in rats exposed prenatally and postnatally to low doses of mercury. Bull Environ Contam Toxicol 13:73-79. Reviewed in Greenman et al. 1980.

Patty's Industrial Hygiene and Toxicology. 1981a. Ethers. Vol. 2P. New York: Wiley-Interscience. p. 2536.

Patty's Industrial Hygiene and Toxicology. 1981b. Phenols and phenolic compounds. Vol. 2A. New York: Wiley-Interscience. p. 2588.

Pelissier MA, Albrecht R. 1976. Teneur minimale du regime en lindane induisant les monoxygenases microsamales chez le rat. Food Cosmet Toxicol 14:297-301. Reviewed in Greenman et al. 1980.

Perry HM Jr., Erlanger MW. 1974. Metal-induced hypertension following chronic feeding of low doses of cadmium and mercury. J Lab Clin Med 83:541-547. Reviewed in Greenman et al. 1980.

Pyzybylski W. 1975. Formation of aflatoxin derivatives on thin layer chromatographic plates. J Assoc Off Anal Chem 58:163-164. Reviewed in Greenman et al. 1980.

Ralston Purina Co. (n.d.) Life cycle information data sheet. Checkerboard Square, St. Louis, MO.

Ralston Purina Co. 1980. Certified rodent chow #5002 specifications, guinea pig chow #5026 specifications, rabbit chow #5322 specifications, and canine diet #5007 specifications. Checkerboard Square, St. Louis MO.

Rogers AE. 1975. Variable effects of a lipobiope-deficient high-fat diet on chemical carcinogenesis in rats. Cancer Res 35:2469-2474.

Rogers AE, Sanchez O, Feinsod FM, Newberne PM. 1974. Dietary enhancement of nitrosamine carcinogenesis. Cancer Res 34:96-99.

Sadler PJ. 1982. Inorganic pharmacology. Chemistry in Britain 18(8):182-188.

Saffioti U, Montesano R, Sellakumar AR, Borg SA. 1967. Experimental cancer of the lung: inhibition of vitamin A of the induction of tracheobronchial squamous metaplasia and squamous cell tumors. Cancer 20:857-864.

Schoental R. 1981. Dietary influence on cancer: traditional and modern. Workshop on fat and cancer. Cancer Res 41:3678.

Schrauzer GN, Ishmael D. 1974. Effects of selenium and of arsenic on the genesis of spontaneous mammary tumors in inbred C3H mice. Ann Clin Lab Sci 4:441-447. Reviewed in Greenman et al. 1980.

Schrauzer GN. 1977. Trace elements, nutrition and cancer: perspectives of prevention. In: Inorganic and nutritional aspects of cancer. Schrauzer GN, ed. New York: Plenum. pp. 323-344.

Schrauzer GN, White DA, Schneider CJ. 1977. Cancer mortality correlation studies - IV: Association with dietary intakes and blood levels of certain trace elements, notably Se-antagonists. Bioinorganic Chem 7:35-56.

Schroeder HA, Balassa JJ. 1967. Arsenic, germanium, tin and vanadium in mice: Effects on growth, survival and tissue levels. J Nutr. 92:245-252. Reviewed in Greenman et al. 1980.

Schroeder HA, Balassa JJ, Vinton WH Jr. 1964. Chromium, lead, cadmium, nickel and titanium in mice: Effects on mortality, tumors and tissue levels. J Nutr 83:239-250. Reviewed in Greenman et al. 1980.

Schroeder HA, Balassa JJ, Vinton WH Jr. 1965. Chromium, cadmium and lead in rats: Effects on life span, tumors and tissue levels. J Nutr 86:51-66. Reviewed in Greenman et al. 1980.

Schroeder HA, Mitchener M. 1971a. Toxic effects of trace elements on the reproduction of mice and rats. Arch Environ Health 23:102-106. Reviewed in Greenman et al. 1980.

Schroeder HA, Mitchener M. 1971b. Selenium and tellurium in rats: Effect on growth, survival and tumors. J Nutr 101:1531-1540. Reviewed in Greenman et al. 1980.

Schroeder HA, Mitchener M. 1972. Selenium and tellurium in mice: Effects on growth, survival, and tumors. Arch Environ Health 24:66-71. Reviewed in Greenman et al. 1980.

Shabad LM, Kolesnickenko TS, Nikonova TV. 1973. Transplacental and combined long-term effect of DDT in five generations of A-strain mice. Int J Cancer 11:688-693. Reviewed in Greenman et al. 1980.

Shelton DC. 1982. Personal communication to B.C. Pal. Ralston Purina Company. Checkerboard Square, St. Louis, MO 63188.

Shendinkar AD. 1982. Trace elements and cancer. Chem Eng News, Jan 18, p. 4.

Shinozuka H, Katyal SL, Lombardi B. 1978. Azaserine carcinogenesis: organ susceptibility change in rats fed a diet devoid of choline. Int J Cancer 22:36-39.

Silinskas KC, Okey AB. 1975. Protection by 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) against mammary tumors and leukemia during prolonged feeding of 7,12-dimethylbenze(a)anthracene to female rats. J Natl Cancer Inst 55:653-657. Reviewed in Greenman et al. 1980.

Silverman J, Adams JD. 1983. N-nitrosamines in laboratory animal feed and bedding. Lab Animal Sci 33(2):161-164.

Silverstone H, Tannenbaum A. 1951. Proportion of dietary protein and the formation of spontaneous hepatomas in the mouse. Cancer Res 11:442-446.

Smith JC, Brown ED, McDaniel EG, Chan W. 1976. Alterations in vitamin A metabolism during Zn deficiency and food and growth restriction. J Nutr 106:569.

Smith DM, Rogers AE, Herndon BJ, Newberne PM. 1975. Vitamin A (Retinyl Acetate) and Benzo(a)pyrene-induced respiratory tract carcinogenesis in hamsters fed a commercial diet. Cancer Res 35:11-16.

Smith DM, Rogers AE, Newberne PM. 1975. Vitamin A and benzo(a)pyrene carcinogenesis in the respiratory tract of hamsters fed a semisynthetic diet. Cancer Res 35:1485-1488.

Smith JC, McDaniel EG, Fan FF, Halsted JA. 1973. Zinc: a trace element essential in vitamin A metabolism. Science 181:954.

Sparnins VL, Venegas PL, Wattenberg LW. 1982. Glutathione S-transferase activity: enhancement by compounds inhibiting chemical carcinogensis and by dietary constituents. J Nat Cancer Inst 68:493-495.

Sterba JP. 1982. National study finds lead levels in blood have declined sharply. The New York Times, March 20, p. 8.

Stob M. 1973. Estrogens in Foods. In: Toxicants occurring naturally in foods, 2nd ed., National Academy of Sciences, Washington, DC.

Stubblefield RD, Shotwell OL, Hesseltine CW, Smith ML, Hall HH. 1967. Production of aflatoxin on wheat and oats: Measurement with a recording densitometer. Appl Microbiol 15:186-190. Reviewed in Greenman et al. 1980.

Swann PF, McLean AEM. 1968. The effect of diet on the toxic and carcinogenic action of dimethylnitrosamine. Biochem J 107:14-15.

Swithin A, Yagi K. 1958. Distribution in renal cell functions of sulfhydryl groups in rats on normal and sucrose diets and its relation to renal mercury distribution after mercuric chloride injection. Am J Physiol 192:405-409.

Tarjan R, Kemeny T. 1969. Multigeneration studies on DDT in mice. Food Cosmet Toxicol 7:215-222. Reviewed in Greenman et al. 1980.

Terracini B, Testa MC, Cabral JR, Day N. 1973. The effects of long-term feeding of DDT to BALB/c mice. Int J Cancer 11:747-764. Reviewed in Greenman et al. 1980.

Tomatis L, Turusov V, Day N, Charles RT. 1972. The effect of long-term exposure to DDT on CF-1 mice. Int J Cancer 10:489-506. Reviewed in Greenman et al. 1980.

Ueno Y. 1980. Trichothecene mycotoxins mycology, chemistry, and toxicology. Adv Nutr Res 3:301-353.

Underwood EJ. 1977. Trace elements in human and animal nutrition. New York: Academic.

USEPA. 1969. U.S. Environmental Protection Agency. Chemistry laboratory manual bottom sediments. Chicago: Great Lakes Region. pp. 38-41. Reviewed in Greenman et al. 1980.

USEPA. 1979. U.S. Environmental Protection Agency. Part IV. Proposed health effects test-standards for toxic substances control act test rules and proposed good laboratory practice standards for health effects. Appendix B. Dietary requirements and contaminant analysis.

USEPA. 1981. U.S. Environmental Protection Agency. Tolerances and exemptions from tolerances for pesticide chemicals in or on raw agricultural commodities. (40CFR180).

USFDA. 1980a. U.S. Food and Drug Administration. Tolerances for pesticides in animal feeds administered by the Environmental Protection Agency. (21CFR561).

USFDA. 1980b. U.S. Food and Drug Administration. Compliance policy guide 7126.33 chapter 26 - animal feed. 10.01.80.

USFDA. 1981. U.S. Food and Drug Administration. Compliance policy guide 7126.27, attachment D, chapter 26 - animal feed. 07.01.81.

Virgo BB, Bellward GD. 1975. Effects of dietary dieldrin on reproduction in the Swiss Vancouver (SWV) mouse. Environ Physiol Biochem 5:440-450. Reviewed in Greenman et al. 1980.

Walker AIT, Stevenson DE, Robinson J, Thorpe E, Roberts M. 1969. The toxicology and pharmacodynamics of dieldrin (HEOD): Two-year oral exposure of rats and dogs. Toxicol Appl Pharmacol 15:345-373. Reviewed in Greenman et al. 1980.

Walker AIT, Thorpe E, Stevenson DE. 1972. The toxicology of dieldrin (HEOD). I. Long-term oral toxicity studies in mice. Food Cosmet Toxicol 11:415-432. Reviewed in Greenman et al. 1980.

Wattenberg LW. 1972. Inhibition of carcinogenic and toxic effects of polycyclic aromatic hydrocarbons by phenotic antioxidants and ethoxyquin. J Natl Cancer Inst 48:1425-1430.

Wattenberg LW. 1975. Effects of dietary constituents on the metabolism of chemical carcinogens. Cancer Res 35:3326-3331.

Wattenberg LW. 1979. Inhibitors of chemical carcinogens. In: Environmental carcinogenesis, Emmelot P., Krizek E, eds. Amsterdam: Elsevier.

Wattenberg LW. 1980. Inhibitors of chemical carcinogens. In: Cancer - 1980: Achievements, challenges, prospects, 1980 International Symposium, Memorial Sloan-Kettering Cancer Center and N.C.I. New York. Grune and Stratton.

Wattenberg LW. 1983. Inhibition of neoplasia by minor dietary constituents. Cancer Research (Suppl.) 43:2448s-2453s.

Weinbruch RH, Makinodan T. 1981. Dietary restriction and its effects on immunity and aging. In: Nutrition in the 1980s: constraints on our knowledge. Prog in Clin and Biol Res 67:319-325. Selvey N, White PL, eds. New York.

Weindruch R, Walford RL. 1982. Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. Science 215:1415.

Weisberger AS, Suhrland LG. 1956. Studies on analogues of L-cysteine and L-cystine. Blood 11:11.

Weisse I, Herbst M. 1977. Carcinogenicity study of lindane in the mouse. Toxicology 7:233-238. Reviewed in Greenman et al. 1980.

Wiebel FJ, Gelboin HV, Buu-Hoi NP, Stout MG, Burnham WS. 1974. Flavones and polycyclic hydrocarbons as modulators of aryl hydrocarbon [Benzo(a)pyrene] hydronylase. In: Chemical carcinogenesis, Part A, Tso POP, DiPaolo JA, eds New York: Marcel Dekker.

Wilson BJ, Hayes AW. 1973. Microbial toxins. In: Toxicants occurring naturally in foods. Natl Acad Sci, Washington, DC. pp. 372-423.

Wogan GN, Newberne PM. 1967. Dose-response characteristics of aflatoxin B_1 carcinogenesis in the rat. Cancer Res 27:2370-2376. Reviewed in Greenman et al. 1980.

Wogan GN, Paglialunga S, Newberne PM. 1974. Carcinogenic effects of low dietary levels of aflatoxin B_1 in rats. Food Cosmet Toxicol 12:681-685. Reviewed in Greenman et al. 1980.

Wyllie TD, Morehouse LG, eds. 1977. Mycotoxic fungi, mycotoxins, mycolotoxicoses, Vol. 1, p. 392, Marcel Dekker, NY.

APPENDIX A. NATIONAL INSTITUTES OF HEALTH STANDARD FOR NUTRIENT AND CHEMICAL CONTAMINANT ANALYSES OF LABORATORY ANIMAL DIETS

NIH STD No. 5A
November 1, 1980
Superseding NIH STD No. 5
November 1, 1978

1. Scope

1.1 This specification covers nutrient and chemical contaminant analyses on samples of laboratory animal diets purchased under NIH contracts.

2. Requirements

2.1 Laboratory analyses to be performed

a. Nutrient Analyses - Diet samples submitted shall be subjected to the following nutrient analyses in accordance with the most recent issue of Official Methods of Analyses of the Association of Official Analytical Chemists (A.O.A.C.).

(1) Moisture
(2) Crude Protein
(3) Crude Fat
(4) Ash
(5) Crude Fiber
(6) Nitrogen-Free-Extract
(7) Calcium
(8) Phosphorus

Nutrient analyses shall be conducted on duplicate samples.

b. Contaminant Analyses - Diet samples submitted shall be subjected to the following contaminant analyses in accordance with the indicated procedure.

Contaminant	Analysis Method
(1) Chlorinated hydrocarbon pesticides (2) Polychlorinated biphenyls (PCB's). (3) Organo-phosphate pesticides.	Shall be conducted in accordance with A.O.A.C. section 29.011 for sample preparations. Electron capture detection by gas liquid chromatography.
(4) Lead	Shall be conducted in accordance with A.O.A.C. section 25.068 for sample preparation; analysis by atomic absorption.

Contaminant	Analysis Method
(5) Arsenic	Shall be conducted in accordance with Anal. Chem. 48: Page 120, 1976 for sample preparations (discussion on automation may be disregarded). Analysis by atomic absorption.
(6) Cadmium	Shall be conducted in accordance with A.O.A.C. section 25.105 for sample preparation; analysis by atomic absorption using a double beam instrument with simultaneous background correction.
(7) Mercury	Shall be conducted in accordance with A.O.A.C. section 25.105 for sample preparation; analysis by atomic absorption.
(8) Aflatoxins	Shall be conducted in accordance with A.O.A.C. section 16.A01-26.A08 (mini column method for combined B_1, B_2, G_1, G_2).
(9) Nitrate	Shall be conducted in accordance with J.A.O.A.C., 51, P. 763, 1968.

Contaminant analyses on duplicate samples is not required. Samples to be analyzed under this Specification will be submitted by the NIH animal feed contractors and/or the project officer. The feed manufacturers and the Independent Laboratory performing the analyses will be notified in writing, at the time the contract is awarded, of the diet samples which they are authorized to submit for analyses. The Government will be responsible to pay the cost of analyzing only these authorized samples.

2.2 Frequency of Assays - All specified nutrient and contaminant analyses shall be completed within 28 days after the feed samples are received by the laboratory. The laboratory shall maintain a log indicating the date each sample is received, the name of the company submitting the sample and the complete identification. (Feed contractors are required to identify all samples with the name of the diet, the date of manufacture and the NIH stock number.)

APPENDIX B. CONVERSION FACTORS FOR SELECTED VITAMINS

---

[a]1 IU of Vitamin A = 1 USP unit
= 0.3 µg all-*trans* retinol
= 0.344 µg all-*trans* retinyl acetate or
0.550 µg all-*trans* retinyl palmitate
= 0.6 µg of all-*trans* β-carotene

[a]1 mg of β-carotene = 1667 IU of Vitamin A
= 833 IU of Vitamin A activity for the dog

[b]1 IU of Vitamin D = 0.025 µg of cholecalciferol

[b]1 IU of Vitamin E = 1 mg of dl-α-tocopheryl acetate
= 0.91 mg of dl-α-tocopherol

[b]1 mg of thiamine HCl = 0.89 mg of thiamine

[b]1 mg of calcium pantothenate = 0.92 mg of pantothenic acid

[b]1 mg of pyridoxine HCl = 0.82 mg of pyridoxine

[b]1 mg of Vitamin K^1 = 0.73 mg menadione sodium bisulfite
or 0.38 mg menadione

Source: [a]NAS (1974).
[b]Merck Index (1968).

APPENDIX C. LETTER FROM EDWARD J. BALLITCH

April 8, 1982

Dr. B. C. Pal
Oak Ridge National Laboratory
Post Office Box x
Oak Ridge, Tennessee 37830

Dear Dr. Pal

This responds further to your telephone call of March 23, 1982, to Dr. Gerald Guest, Acting Bureau Director, and our telephone conversation of March 32, 1982, asking whether the Delaney anticancer clause in the Federal Food, Drug and Cosmetic Act (Act) is applicable to selenium incorporated into commercially prepared laboratory animal feed.

This clause, in section 409(c)(3)(A) of the Act (21 U.S.C. 348(c)(3)(A)), prohibits issuance of a food additive regulation for a substance which induces cancer, except that it does not apply with respect to an ingredient of feed for food producing animals under specified conditions. Therefore, under a literal reading of the law, an article which is a carcinogen may not be approved for use in laboratory animal feed, as you know, selenium has been demonstrated to induce cancer in laboratory animals.

The agency recognizes, however, that selenium is an essential nutritional element for laboratory animals and that feed is the most practical method of providing this nutrient. Furthermore, its use for this purpose is very limited and carefully monitored in laboratory animal diets. We have, therefore, concluded that our current position is that we do not intend to take regulatory action against the marketing and use of appropriate supplemental sources of selenium at generally accepted nutritional levels in laboratory animal feeds solely on the basis that a regulation for this use is prohibited under the Act. Of course, if future information raises safety concerns regarding this policy, the agency will take appropriate measures.

Undoubtedly you are aware of data suggesting that trace levels of selenium act to inhibit carcinomas in laboratory animals. We caution that this fact be taken into account when designing toxicity studies involving animals fed selenium in their feed. Selenium levels in laboratory animal feed should be reported in the study results.

Edward J. Ballitch
Director
Division of Compliance
Bureau of Veterinary Medicine
Food and Drug Administration
Rockville, MD 20857

APPENDIX D. ABBREVIATIONS LIST

AIN	American Institute of Nutrition
BHA	Butylated hydroxyanisole
IU	International units
NAS	National Academy of Sciences
NDMA	N-Nitrosodimethylamine
NIH	National Institutes of Health
NRC	National Research Council
TDN	Total Digestible Nutrients
USDA	United States Department of Agriculture
USP	United States Pharmacopoeia

APPENDIX E. ADDITIONAL LITERATURE REFERENCES

Aaron A, Schad JS. 1954. Nitrogen balance studies with dogs on casein or methionine supplemented casein. J Nutr 53:265.

Ackerman CJ. 1957. Reversal of sulfaguanidine toxicity in the rat. J Nutr 63:131-42.

Afonsky D. 1954. Folic acid deficiency in the dog. Science 120:803.

Agar J. 1971. Effect of a low zinc diet during gestation on reproduction in the rabbit. J Anim Sci 33:1255.

Ahlborg UG, Dich J. 1978. The use of food additives in Sweden. Arch Toxicol Suppl 1:305-308.

American Association for Cancer Research. 1981. Workshop on fat and cancer. Cancer Res 41(9):3677-3826, part 2 of 2.

American Institute of Nutrition Ad Hoc Committee Report on Standards for Nutritional Studies. 1977. J Nutr 107(7):1340-1348.

Anonymous. 1978. BRIEFING: Nitrosamines found in NIH-approved animal feed. Science 202:192.

Arnich L, Lewis EM, Morgan AF. 1952. Growth of dogs on purified diet plus aureomycin and/or vitamin B_{12}. Proc Soc Exp Biol Med 80:401.

Arrington LR, Taylor RN Jr, Ammerman CB, Shirley, RL. 1965. Effects of excess dietary iodine upon rabbits, hamsters, rats and swine. J Nutr 87:394.

Asburn LL. 1940. The effects of administration of pantothenic acid on the histopathology of the filtrate factor deficiency state in rats. Public Health Rep 55:1337.

Baggs RB, Miller SA. 1973. Nutritional iron deficiency as a determinant of host resistance in the rat. J Nutr 103:1554.

Balazs T, Sekella R, Pauls JF. 1971. Renal concentration test in beagle dogs. Lab Anim Sci 21:546.

Balk MW, Foster HL. 1982. Histocompatibility and isoenzyme differences in commercially supplied BAL/c mice: A reply. Science 217:381.

Ballitch EJ. 1982. Personal communication. Letter dated 4/8/82 to Dr. B.C. Pal.

Barki VH, Derse PH, Collins RA, Hart EB, Elvehjem CA. 1949. The influence of coprophagy on the biotin and folic acid requirements of the rat. J Nutr 37:443.

Barnes LL, Sperling G, Maynard LA. 1941. Bone development in the albino rat on a low manganese diet. Proc Soc Exp Biol Med 46:562.

Barnes RH, Bates MJ, Maack J. 1946. The growth and maintenance utilization of dietary protein. J Nutr 32:535.

Barnes RH, Fiala G. 1959. Effects of the prevention of coprophagy in the rat. VI. Vitamin K. J Nutr 68:603.

Barnes RH, Lundberg, WO, Hansen HT, Burr GO. 1943. The effect of certain dietary ingredients on the keeping quality of body fat. J Biol Chem 149:313.

Barney GH, Macapinlac MP, Pearson WN, Dalby WJ. 1967. Parakeratosis of the tongue-a unique pathologic lesion in the zinc deficient squirrel monkey. J Nutr 93:511.

Barstad. 1978. Pesticides and heavy metals as food contaminants. Arch Toxicol Suppl 1:47-54.

Baur LS, Filer LJ, Jr. 1958. Evaluation of lysine supplementation of infant formulas fed to rats. J Nutr 66:545-54.

Beard HH. 1929. Studies in the nutrition of the white mouse. V. The experimental production of rickets in mice. Am J Physiol 89:54.

Beaton JR, Beare JL, McHenry EW. 1952. Factors affecting the development acrodynia in pyridoxine-deficient rats. J Nutr 48:325.

Beck EM, Fenton PF, Cowgill GR. 1950. The nutrition of the mouse. IX. Studies on pyridoxide and thiouracil. Yale J Biol Med 23:190.

Behne, D, Hoer T, von Berswordt-Wallrabe R, Elger W. 1982. Selenium in the testis of the rat: studies on its regulation and its importance for the organism. J Nutr 112:1682-1687.

Bender AD, Schottelium DD, Schottelius BA. 1959. Effect of short-term vitamin E deficiency on guinea pigs, skeletal muscle myoglobin. Am J Physiol 197:491.

Bennett MJ, Coon E. 1966. Mellituria and post-prandial blood sugar curves in dogs after the ingestion of various carbohydrates with the diet. J Nutr 80:163-168.

Berg BN, Simms HS. 1961. Nutrition and longevity in the rat. III. Food restriction beyond 800 days. J Nutr 74:23.

Bergman EN, Sellers AF. 1960. Comparison of fasting ketosis in pregnant and nonpregnant guinea pigs. Am J Physiol 198:1083.

Bessey OA, Wolbach SB. 1939 Vascularization of the cornea of the rat and riboflavin deficiency with a note on corneal vascularization and vitamin A deficiency. J Exp Med 69:1.

Best CH, Huntsman ME, Solandt OM. 1932. A preliminary report on the effect of choline on fat deposition in species other than the white rat. Trans R Soc Can 26:175.

Best CH, Huntsman ME. 1932. The effects of the component of lecithin upon deposition of fat in the liver. J Physiol 75:405.

Best CH, Hershey JM, Huntsman ME. 1932. Effect of lecithin on fat deposition in the liver of the normal rat. J Physiol 75:56.

Bethke RM, Kick CH, Wilder W. 1932. The effect of calcium-phosphorus relationship on growth, calcification, and blood composition of the rat. J Biol Chem 98:389.

Bild CE. 1959. Raising healthy pups. Proc Animal Care Panel 9:79-82.

Bloodworth JMB. 1965. Experimental diabetic glomerulosclerosis. II. The dog. Arch Pathol 79:113.

Bloom WL, Azar GJ. 1963. Similarities of carbohydrate deficiency and fasting. Arch Intern Med 112:333.

Boas MA. 1927. An observation on the value of egg white as a sole source of nitrogen for young growing rats. The effect of dessication upon the nutritive properties of egg white. Biochem J 21:712.

Boelter MDD, Greenberg DM. 1943. Effect of severe calcium deficiency on pregnancy and lactation in the rat. J Nutr 26:105.

Boffey PM. 1982. In wake of mouse mix-up, studies are left in ruin assessing the effects of mixup in lab mice. The NY Times, July 27, 1982.

Borchers R. 1958. Effect of dietary level of raw soybean oil meal on the ground of weanling rats. J Nutr 66:229-233.

Bosshardt DK, Paul WJ, Barnes RH. 1950. The influence of diet composition on vitamin B_{12} activity in mice. J Nutr 40:595.

Bouman J, Slater EC. 1956. Tocopherol content of heart muscle preparations. Nature 177:1181.

Bourne GH. 1957. Some aspects of the feeding of dogs and other carnivora. Laboratory Animals Bureau, MRC Laboratories, London. Collected papers 5:81-87.

Braham JE, Villarreala A, Bressani R. 1962. Effect of lime treatment of corn on the availability of niacin for cats. J Nutr 76:183.

Bras G, Ross MH. 1964. Kidney disease and nutrition in the rat. Toxicol Appl Pharmacol 6:247.

Brody JE. 1982. A sizeable number of Americans may not get enough zinc. The NY Times, July 28, 1982.

Brown D, Ferguson JD, Ramsay AG. 1953. Guinea-pigs reared on a diet containing synthetic ascorbic acid. J Physiol (London) 121:36-37P.

Brown EB, Smith DE, Dubach R, Moore CB. 1959. Lethal iron overload in dogs. J Lab Clin Med 53:591.

Browning CB, Parrish DB, Fountaine FC. 1958. Effect of feeding low levels of diethylstilbestrol on gestation and lactation of rats. J Nutr 66:321-322.

Bryan WL, Mason KE. 1940. Vitamin E deficiency in the mouse. Am J Physiol 131:263.

Bryan GT, Brown RR, Price JM. 1964. Incidence of mouse bladder tumors following implantation of paraffin pellets containing certain tryptophan metabolites. Cancer Res 24:582.

Buckley GP, Hartroft WS. 1955. Pathology of choline deficiency in the mouse-observations with special reference to liver. Arch Pathol 59:185.

Bull LS, Hurley WL, Kennett WS, Tamplin CB, Williams WF. 1974. Effect of sex hormones on feed intake in rats. J Nutr 104:968.

Bunce GE, Jenkins KS, Phillips PH. 1962. The mineral requirements of the dog. III. The magnesium requirement. J Nutr 76:17.

Burr GO, Burr MM. 1930. On the nature and role of the fatty acids essential in nutrition. J Biol Chem 86:587.

Campbell HL. 1945. Seasonal changes in food consumption and rate of growth of the albino rat. Am J Physiol 143:428-433.

Campbell JE, Phillips PH. 1953. Some problems on feeding fat to dogs. Southwest Vet 6:173.

Campbell TC. 1981. A decision tree approach to the regulation of food chemicals associated with irreversible toxicities. Regulatory Toxicology and Pharmacology 1:193-201.

Cantor AH, Longevin ML, Noguchi T, Scott ML. 1975. Efficacy of selenium in selenium compounds and feedstuffs for prevention of pancreatic fibrosis in chicks. J Nutr 105:106-111.

Cantor AH, Scott ML. Noguchi T. 1975. Biological availability of selenium in feedstuffs and selenium compounds for prevention of exudative diathesis in chicks. J Nutr 105:96-105.

Carpenter KJ, Kodicek E. 1948. The blood picture in pyridoxin and riboflavin deficiencies in rats. Br J Nutr 2:ix.

Carroll KK, Khor HT. 1971. Effects of level and type of dietary fat on incidence of mammary tumors induced in female Sprague-Dawley rats by 7,12-dimethylbenz(α)anthracene. Lipids 6:415.

Cassidy J. 1957. The commercial manufacture of compressed diets for laboratory animals. Laboratory Animals Bureau, MRC Laboratories, London. Collected Papers 5:15-19.

Chapin RE, Smith SE. 1967. Calcium requirement of growing rabbits. J Anim Sci 26:67.

Chesney AM, Clawson TA, Webster B. 1928. Endemic goitre in rabbits. 1. Incidence and characteristics. Bull John Hopkins Hosp 32:261.

Christensen F, Dam H. 1953. A new symptom of fat deficiency in hamsters: profuse secretion of cerumen. Acta Physiol Scand 27:204.

Cohen ML, Reyes P, Typpo JT, Briggs GM. 1967. Vitamin B_{12} deficiency in the golden hamster. J Nutr 91:482.

Cohen ML, Arnrich L, Okey R. 1963. Pantothenic acid deficiency in cholesterol-fed hamsters. J Nutr 80:142.

Cole VV, Harned BK. 1938. Diabetic traits in a strain of rats. Endocrinology 23:318.

Committee on Animal Nutrition. 1966. Nutrient requirements of domestic animals. Biological Energy Interrelationships and Glossary of Energy Terms. 1st revised edition 1966.

Cooperman JM, Waisimen HA, Elvehjem CA. 1943. Nutrition of the hamster. Proc Soc Exp Biol Med 52:250-254.

Coots MC, Harper AE, Elvehjem CA. 1959. Production of biotin deficiency in the guinea pig. J Nutr 67:525.

Corbin J. 1976. Laboratory animal nutrition. In: CRC handbook on laboratory animal science. Melby GC Jr, Actman NH, eds. Vol. 3.

Cordes DO, Mosher AH. 1966. Brown pigmentation (lipofuscinosis) of the canine intestinal muscularis. J Pathol Bacteriol 92:197.

Cotes PM, Reid E, Young FG. 1949. Diabetogenic action of pure anterior pituitary growth hormone. Nature 164:209.

Cowan JC. 1978. Soybean. In: Kirk-Othmer encyclopedia of chemical technology. John Wiley & Sons, New York.

Cowgill GR. 1921. A contribution to the study of the relation between vitamin B and the nutrition of the dog. Am J Physiol 57:420.

Crampton EW. 1964. Nutrient to calorie ratios in applied nutrition. J Nutr 82:353.

Crimm PD, Short DM. 1937. Vitamin A deficiency in the dog. Am J Physiol 118:477.

Cusack RP, Brown WD. 1965. Iron deficiency in rats: changes in body and organ weights, plasma proteins, hemoglobins, myoglobins and catalae. J Nutr 86:383.

Cuthbertson WFJ. 1957. The nutritional requirements of rats and mice. Laboratory Animals Bureau, MRC Laboratories. London. Collected Papers 5:27-37.

Czajka-Narins DM, Miller SA, Browning, AM. 1973. Studies of the protein requirement of the neonatal rat. J Nutr 103:1608.

Daft FS, Schwarz K. 1952. Prevention of certain B vitamin deficiencies with ascorbic acid or antibiotics. Fed Proc 11:200.

D'Arcy PV, Howard EM. 1962. Acute toxicity of ferrous salts. J Pathol Bacteriol 83:65.

Day HG, Skidmore BE. 1947. Some effects of dietary zinc deficiency in the mouse. J Nutr 33:27.

DeLuca HF. 1974. Vitamin D: The vitamin and the hormone. Fed Proc 33:2211.

Dowling MT, Fenton PF. 1953. Nutrition of the mouse. XII. Comparison of several salt mixtures. Proc Soc Exp Biol Med 82:16-17.

Draper HH, Csallany AS. 1969. Metabolism and function of vitamin E. Fed Proc 28:1690.

Dupuis Y. 1957. Lactose and utilization of calcium. I. Influence of lactose on calcium retention and skeletal development in the growing rat at various diets. Compt Rend Soc Biol 151:1722-1725.

Efremov VV, Makarychev AI, Tikhomirova AN. 1954. Effects of niacin avitaminosis on conditioned reflexes in dogs, Vopr. Pitan, 13:10, Chem Abstr 49:11800.

Ellinger F. 1956. Water intake of guinea pigs as parameter in oral drug therapy of experimental irradiation. Proc Soc Exp Biol Med 91:172-174.

Elliott RF, Whitehill AR. 1957. Placental and mammary transfer of ingested chlortetracycline in the rat. Proc Soc Exp Biol Med 94:119-122.

Ellis GH, Smith SE, Gates EM. 1947. Further studies of manganese deficiency in the rabbit. J Nutr 34:21.

Epstein SH, Morgulis S. 1941. The minimum requirement of rabbits for dl-α-tocopherol. J Nutr 22:415.

Erickson BA, Odell BL. 1961. Major dietary constituents and vitamin B_{12} requirement. J Nutr 75:414.

Ershoff BH. 1956. Beneficial effects of alfalfa aureomycin and cornstarch on the growth and survival of hamsters fed highly purified rations. J Nutr 59:579.

Evans HM. 1922. On the existence of the hitherto unrecognized dietary factor essential for reproduction. Science 56:650.

Evans HM, Burr GO. 1928. Development of paralysis in suckling young of mothers deprived of vitamin F. J Biol Chem 76:273.

Everitt AV. 1958. The change in food and water consumption and in feces and urine production in ageing male rats. Gerontologia 2:21-32.

Everson GJ, Hurley LS, Geiger JF. 1959. Manganese deficiency in the guinea pig. J Nutr 68:49.

Everson GJ, Tsai HC, Wang T. 1967. Copper deficiency in the guinea pig. J Nutr 93:533.

Exley SH. 1958. Nutrition of the cat. J Am Vet Med Assoc 132:538-539.

Farm Chemicals Handbook. 1981.

Fenton PF. 1957. Hereditary factors in protein nutrition. Am J Clin Nutr 5:663.

Festing MFW. 1979. Inbred strains in biomedical research. Oxford University Press, NY.

FDA. 1978. Food ¢ Drug Administration. Good laboratory practice standards of the health effects testing standards under Toxic Substances Control Act. Fed Regist 43 Fr(247):59986-60025.

Foley EJ. 1942. Toxemia of pregnancy in the guinea pig. J Exp Med 75:539.

Folk GE, Jr., Farrand RL. 1957. Factors influencing the nutritional status of the hamster. Proc Animal Care Panel 7:245-252.

Follis RH, Jr. 1964. Further observations on thyroiditis and colloid accumulation in hyperplastic thyroid glands of hamsters receiving excess iodine. Lab Invest 13:1590.

Forbes RM. 1964. Mineral utilization in the rat. II. Restoration of normal tissue levels of magnesium and calcium following magnesium deficiency. J Nutr 83:44.

Forbes RM. 1967. Studies of zinc metabolism. New methods of nutrition biochemistry. Vol. III.

Fouts PJ, Helmer OM, Lepkovsky S, Jukes TH. 1938. Production of microcytic hypochromic anemia in puppies on synthetic diet deficient in rat anti-dermatitis factor. J Nutr 16:197.

Fox JG. 1977. Clinical assessment of laboratory rodents on long term bioassay studies. J Environ Pathol Toxicol 1:199-226.

Fox MRS, Mickelsen O. 1959. Salt mixtures for purified-type diets: I. Effect of salts in accelerating oxidative rancidity. J Nutr 67:123.

Frandsen AM, Nelson MM, Suton E, Pecks H, Evans HM. 1954. The effects of various levels of dietary protein on skeletal growth and endochondral ossification in young rats. Anat Rec 119:247.

Fraser DR, Kodicek E. 1970. Unique biosynthesis of kidney of a biologically active vitamin D metabolite. Nature 228:764.

Frazer TW. 1964. Diabetes insipidus in the dog. Vet Rec 76:543.

Freeman GG, Morrison RI. 1949. The isolation and chemical properties of trichothecin, an antifungal substance from trichothecium roseum link. Biochem J 44:1.

Friedman WF, Roberts WC. 1966. Vitamin D and the supravalvar aortic stenosis syndrome. The transplacental effects of vitamin D on the aorta of the rabbit. Circulation 34:77.

From GLA, Craighead JE, McLane MF, Steinke J. 1968. Virus-induced diabetes in mice. Metabolism 17:1158.

Frost D. 1982. Selenium/arsenic vs cancer. Chem Eng News. April 12, 1982.

Frost DV. 1981. In defense of arsenic. Chem Eng News. October 5, 1981, p. 4.

Frost DV, Elvehjem CA, Hart EB. 1940. Iron utilization in dogs on milk diets. J Nutr 19:311.

Gabrielsen BO, Opstvedt J. 1980. Availability of selenium in fish meal in comparison with soybean meal, corn gluten meal and selenomethionine relative to selenium in sodium selenite for restoring glutathione peroxidase activity in selenium-depleted chicks. J Nutr 110:1096-1100.

Gabrielsen BO, Opstvedt J. 1980. A biological assay for determination of availability of selenium for restoring blood plasma glutathione peroxidase activity in selenium-depleted chicks. J Nutr 110:1089-1095.

Ganaway JR, Allen AM. 1971. Obesity prediposes to pregnancy toxemia (ketosis) of guinea pigs. Lab Anim Sci 21:40.

Gepts W, Toussaint D. 1967. Spontaneous diabetes in dogs and cats. Diabetologia 3:249.

Gerber GB, Aldrich GF, Koszalka TR, Gerber G. 1962. Biochemical and autoradiographic studies on DNA metabolism in vitamin E deficient hamsters. J Nutr 78:307.

Gershoff SN, Andrus SB. 1961. Dietary magnesium, calcium, and vitamin B6 and experimental nephropathies in rats: calcium oxalate calculi, apatite nephrocalcinosis. J Nutr 73:308.

Gershoff SN, Andrus SB, Hegsted DM. 1959. The effect of the carbohydrate and fat content of the diet upon the riboflavin requirement. J Nutr 68:75.

Gershoff SN, Andrus SB, Hegsted DM, Lentini E. 1957. Vitamin A deficiency in cats. Lab Invest 6:227.

Gershoff SN, Faragalla FF. 1959. Endogenous oxalate synthesis and glycine, serine, deoxypyridoxine interrelationships in vitamin B6 deficient rats. J Biol Chem 234:2391.

Giaja A. 1952. Food consumption and environmental heat. Compt Rend 235:818:820.

Giroud A, Martinet M. 1962. Smallness of the teratogenic dose of vitamin A. C R Soc Biol 156:449.

Goddard KM, Williams GD, Newberne PM, Wilson RB. 1970. A comparison of all-meat, semimoist, and dry-type dog foods as diets for growing beagles. J Am Vet Med Assoc 157:1233.

Goettsch M. 1930. The dietary production of dystrophy of the voluntary muscles. Proc Soc Exp Biol Med 27:564.

Goettsch MA. 1942. Alpha tocopherol requirement of the mouse. J Nutr 23:513.

Goettsch MA. 1960. Comparative protein requirement of rat and mouse for growth, reproduction and lactation using casein diets. J Nutr 70:307.

Goettsch M, Pappenheimer AM. 1931. Nutritional muscular dystrophy in the guinea pig and rabbit. J Exp Med 54:145.

Goldblatt H, Moritz AR. 1925. Experimental rickets in rabbits. J Exp Med 42:299.

Goss H, Guilbert HR. 1939. The minimum vitamin A and carotene requirement of the rat. J Nutr 18:169.

Graham ER, Telle P. 1967. Zinc retention in rabbits: effects of previous diet. Science 155:691.

Grainger RB, O'Dell BI, Hogan AG. 1954. Congenital malformations as related to deficiencies of riboflavin and vitamin B12, source of protein, calcium to phosphorus ratio, and skeletal phosphorus metabolism. J Nutr 54:33.

Granados H. 1951. Nutritional studies on growth and reproduction of the golden hamster. Acta Physiol Scand 24(Suppl.):87.

Gray JI. 1981. Formation of N-nitroso compounds in foods. ACS Symp Ser 174:165-180.

Green HSN. 1938. Toxemia of pregnancy in the rabbit. II. Etiological considerations with special reference to hereditary factors. J Exp Med 67:369.

Greenberg LD, Moon HD. 1961. Alterations in the blood fatty acids in single and combined deficiencies of essential fatty acids and vitamin B6 in monkeys. Arch Biochem Biophys 94:405.

Greenberg SM, Tucker RG, Heming AE, Mathues JK. 1957. Iron absorption and metabolism. 1. Interrelationship of ascorbic acid and vitamin E. J Nutr 63:19.

Griffin CA, McCay CM, Sperling G, Lloyd RG. 1957. Stock diets for mice, rats, rabbits, dogs and chinchillas. Proc Animal Care Panel 7:212-219.

Gueerant NG, Steel JM. 1958. Some effects of aureomycin and penicillin on thiamine and riboflavin metabolism in growing rats. Proc Soc Exptl Biol Med 98:542-545.

Gyorgy P. 1939. The curative factor (vitamin II) for egg white injury with particular reference to its presence in different foodstuffs and in yeast. J Biol Chem 131:733.

Haas GM, Trueheart RE, Taylor CB, Stumpe M. 1958. An experimental histologic study of hypervitaminosis D. Am J Pathol 34:395.

Haist RE. 1949. Studies in experimental diabetes. Am J Med 7:585.

Hamilton JW, Hogan AG. 1944. Nutritional requirements of the syrian hamster. J Nutr 27:213-224.

Handler P, Bernheim F. 1949. Choline deficiency in the hamster. Proc Soc Exp Biol Med 72:569.

Harper AE, Spivey HE. 1958. Relationship between food intake and osmotic effect of dietary carbohydrate. Am J Physiol 193:483-487.

Harrington DD, Newberne PM. 1970. Correlation of maternal blood levels of vitamin A at conception and the incidence of hydrocephalus in newborn rabbits: an experimental animal model. Lab Anim Care 20:675.

Hartroft WS, Best CH. 1947. Lipoid substance in the cells of proximal convoluted tubules of the kidneys of young rats on a choline-deficient diet. Science 105:315.

Hartroft WS, Parta EA. 1968. Alcohol, diet and experimental hepatic injury. Can J Physiol Pharmacol 46:464.

Hayashi K, Boucher DW, Notkins AL. 1974. Virus-induced diabetes mellitus. II. Relationship between beta cell damage and hyperglycemia in mice infected with encephalomyocarditis virus. Am J Pathol 75:91.

Hayes KC, Nielson SW, Rousseau JE, Jr. 1969. Vitamin E deficiency and fat stress in the dog. J Nutr 99:196.

Heard CRC, Turner MR, Platt BS. 1964. Diabetic-like changes in carbohydrate metabolism of dogs fed diets of suboptimal protein value. Proc Nur Soc 23:VI.

Hedhammer AKE, Fu-ming WV, Krook L. 1947. Over-nutrition and skeletal disease: an experimental study in growing Great Dane dogs. Cornell Vet 64(suppl 5):115.

Hendricks JB, Morgan AF, Fretag RM. 1947. Chronic moderate hypervitaminosis D in young dogs. Am J Physiol 149:319.

Herbut PA, Watson JS, Perkins E. 1946. Hepatic and renal necrosis in alloxan diabetes of rabbits. Arch Pathol 41:516.

Hershey JM. 1930. Substitution of lecithin for raw pancreas in the diet of the depancreatized dog. Am J Physiol 93:657.

Hickman KCD, Kaley MW, Harris PL. 1944. Co-vitamin studies. I. The sparing action of natural tocopherol concentrations on vitamin A. J Biol Chem 152:303.

Hoffbauer, FW, Zaki FG. 1965. Choline deficiency in baboon and rat compared. Arch Pathol 79:364.

Hogan AG, Hamilton JW. 1942. Adequacy of diets for guinea pigs and rabbits. J Nutr 23:533-543.

Hogue DE, Hintz HP, Walker EF, Jr, Banefjee GC. 1970. Vitamin E antagonism of kidney beans. Cornell Nutr Conf.

Hotzel D, Barnes RH. 1966. Contributions of intestinal microflora to the nutrition of the host. Vitam Horm 24:115.

Hove EL, Herndon JF. 1955. Potassium deficiency in the rabbit as a cause of muscular dystrophy. J Nutr 55:363.

Hove EL, Copeland DH, Salmon WD. 1954. Choline deficiency in the rabbit. J Nutr 53:377.

Hove EL, Herndon JF. 1957. Vitamin B_6 deficiency in rabbits. J Nutr 61:127-136.

Hunt CE, Carlton WW. 1965. Cadiovascular lesions associated with experimental copper deficiency in the rabbit. J Nutr 87:385.

Hunt CE, Carlton WW, Newberne PM. 1966. Copper deficiency in rats. Fed Proc 25:432.

Hunt CE, Harrington DD. 1974. Nutrition and nutritional diseases. In: The biology of the laboratory rabbit. Weisbroth SH, Flatt RE, Kraus AL, eds. Academic Press, New York.

Hurley LS, Wooten E, Everson GJ. 1961. Disproportionate growth in offspring of manganese-deficient rats. 2. Skull, brain and cerebrospinal fluid. J Nutr 74:282.

Hurley LS, Swenerton H. 1966. Congenital malformations resulting from zinc deficiency in rat. Proc Soc Exp Biol Med 123:692.

Innes JRM, Yevich PP. 1954. So-called nutritional muscular dystrophy as a cause of "paralysis" in rabbits. Am J Pathol 30:555.

ILAR News. 1976. A report of the committee on long-term holding of laboratory rodents. ILAR News, Vol. XIX, Number 4.

Jaffee WG. 1952. Influence of cobalt on reproduction of mice and rats. Science 115:265.

Jaffee WG. 1956. Requirements of rats for vitamin B_{12} during growth, reproduction and lactation. J Nutr 59:135.

Jolly RD, Blakemore WF. 1973. Inherited lysosomal storage diseases: an essay in comparative medicine. Vet Rec 92:391.

Jones CC, Brown SO, Richardson LR, Sinclair JG. 1955. Tissue abnormalities in newborn rats from vitamin B_{12} deficient mothers. Proc Soc Exp Biol Med 90:135.

Jones JH. 1945. Experimental rickets in the hamster. J Nutr 30:143.

Jones JH, Foster C, Dorfman F, Hunter GL. 1945. Effects on the albino mouse of feeding diets very deficient in each of several vitamin B factors (thiamine, riboflavin, pyridoxine, pantothenic acid). J Nutr 29:127.

Jowsey J and Raisz LG. 1968. Experimental osteoporosis and parathyroid activity. Endocrinology 82:384.

Kahan B, Auerbach R, Alter BJ, Bach FH. 1982. Histocompatibility and isoenzyme differences in commercially supplied "BALB/c" mice. Science 217:379-381.

Kalter H, Warkany J. 1957. Congenital malformations in inbred strains of mice induced by riboflavin-deficient, galactoflavin-containing diets. J Exp Zool 136:531.

Kaplan M, Campbell SL, Johnson L, PapaMichael A, Spaker R, Weinbaum M. 1959. Growth of body weight and manipulation of food motivation. Science 129:1673-1674.

Kawachi T, Hirata Y, Sugimura T. 1968. Enhancement of N-nitrosodiethylamine hepatocarcinogenesis by L-tryptophan in rats. GANN 59:523-525.

King DW. 1964. Comparative effects of certain antioxidants on gentational performance and teratogeny in vitamin E-deficient rats. J Nutr 83:123.

Kirwin CJ Jr, Sandmeyer EE. 1981. Butylated hydroxyanisole. In: Patty's industrial hygiene and toxicology Vol. 2A, 3rd ed. Clayton GD, Clayton FE, eds. John Wiley & Sons, NY, p. 2536.

Kirwin CJ Jr, Sandmeyer EE. 1981. Ethylcellulose. In: Patty's industrial hygiene and toxicology. Vol. 2A, 3rd ed. Clayton GD, Clayton FE, eds. John Wiley & Sons, NY, p. 2556.

Knapka JJ, Smith KP, Judge FJ. 1974. Effect of open and closed formula rations on the performance of three strains of laboratory mice. Lab Anim Sci 24(3):480-487.

Ko KW, Fellers FX, Craig JM. 1962. Observations on magnesium deficiency in the rat. Lab Invest 11:294.

Koch OR, Porta EA, Hartroft WS. 1968. A new approach in the study of chronic alcoholism. III. Role of alcohol versus sucrose of fat-derived calories in hepatic damage. Lab Invest 18:379.

Kodicek E, Murray PDF. 1943. Influence of a prolonged partial deficiency of vitamin C on the recovery of guinea pigs from injury to bones and muscles. Nature 151:395.

Konishi F, McCay CM. 1960. The effect of limited water intake on nutrient utilization. J Nutr 70:528.

Krehl WA, Tepley LJ, Elvehjem CA. 1945. Corn as an etiological factor in the production of a nicotinic acid deficiency in the rat. Science 101:283.

Krook L, Lutwak L, Henrikson P, et al. 1971. Reversibility of nutritional osteoporosis: phytochemical data on bones from an experimental study in dogs. J Nutr 101:233.

Kunkel HO, Pearson PB. 1948. Magnesium in the nutrition of the rabbit. J Nutr 36:657.

Kunkel HO, Pearson PB. 1949. The quantitative requirements of the rat for magnesium. Arch Biochem 18:461.

Lane-Petter W. 1957. Modern ways of feeding laboratory animals. In: The nutrition of laboratory animals. Laboratory Animals Bureau, MRC Laboratories. London. Collected Papers, Vol. 5.

Lang M, Vessell C, Vessell ES. 1976. Environmental and genetic factors affecting laboratory animals; impact on biomedical research. Fed Proc, Vol 35(5), April 1976.

Lee S, Chu FS. 1981. Radioimmunoassay of T-2 toxin in corn and wheat. J Assoc Off Anal Chem 64:156.

Lee, YCP, Visscher MB, King JT. 1962. Role of manganese in vitamin E deficiency in mouse paralysis. Am J Physiol 203:1103.

Leonard MA, Loosli JK. 1962. Animal nutrition, 5th ed., McGraw-Hill Book Company.

Leucke RW, Olman ME, Baltzer BV. 1968. Zinc deficiency in the rat: effect on serum and intestinal alkaline phosphatase activities. J Nutr 94:344.

Levers MK, Stefanik PA, Cole DF. 1959. Effect of thiamine deficiency on canine gastric secretion of acid. Am J Physiol 197:253.

Liebow AA, McFarland WJ, Tennant R. 1941. The effects of potassium deficiency on tumor-bearing mice. Yale J Biol Med 13:523.

Linder M, Todd ME, Hagstrom JWC. 1963. Effects of pancreatectomy on development of atherosclerosis in the rabbit. Proc Soc Exp Biol Med 113:725.

Lippincott SW, Morris HP. 1942. Pathologic changes associated with riboflavin deficiency in the mouse. J Natl Cancer Inst 2:601.

Loosli JK. 1945. Feeding laboratory animals. Ann NY Acad Sci 46, 1:45-75.

Lyons MJ, Faust IM, Hemmes RB, Buskirk DR, Hirsch J, Zabriskie JB. 1982. A virally induced obesity syndrome in mice. Science 216:82-85.

Maddock CL, Wolbach SB, Maddock S. 1949. Hypervitaminosis A in the dog. J Nutr 39:117.

Mameesh MS, Johnson BC. 1958. The effect of penicillin on the intestinal synthesis of thiamine in the rat. J Nutr 65:161-167.

Mannering GJ. 1949. Vitamin requirements of the guinea pigs. Vitam Horm 7:201.

Mansford KRL, Opie L. 1968. Comparison of metabolic abnormalities in diabetes mellitus induced by streptozotocin or by alloxan. Lancet 1:670.

Marchett M, Landi L, Pasquali P. 1966. Effects of biotin on folic acid metabolism in the rat. J Nutr 89:422.

Margolis G, Morgolis LH, Smith SG. 1938. Cure of experimental canine black tongue with optimal and minimal dose of nicotinic acid. J Nutr 16:541.

Mason KE. 1935. Changes in the vaginal epithelium of the rat after vitamin A deficiency. J Nutr 9:735.

Mason KE. 1935. Fetal death, prolonged gestation and difficult parturition in the rat as a result of vitamin A deficiency. Am J Anat 57:303.

Matschiner JT, Doisy EA Jr. 1965. Effect of dietary protein on the development of vitamin K deficiency in the rat. J Nutr 86:93.

Mattill HA, Golumbie C. 1942. Vitamin E, cod liver oil and muscular dystrophy. J Nutr 23:625.

McCance RA, Widdowson EM. 1944. Mineral metabolism. Ann Rev Biochem 13:315.

McCarthy PT, Cerecedo LR. 1952. Vitamin A deficiency in the mouse. J Nutr 46:361.

McCollum EB, Simmons N, Decker JE, Shipley PG. 1922. Studies on experimental rickets. XXI. An experimental demonstration on the existence of the vitamin which promotes calcium deposition. J Biol Chem 53:293.

McCollum EB, Simmons N, Parsons HT, Shipley PG, Park EA. 1921. Studies on experimental rickets. I. The production of rachitis and similar diseases in the rat by deficient diets. J Biol Chem 45:333.

McCoy KFM, Weswig PH. 1969. Some selenium responses in the rat not related to vitamin E. J Nutr 98:383.

McGuire EA, Young VR, Newberne PM, Payne BJ. 1968. Effects of _Salmonella typhimurium_ infection in rats fed varying protein intakes. Arch Pathol 86:60.

McKibbin JM, Black S, Elvehjem CA. 1940. The essential nature of pantothenic acid and another alkali-labile factor in the nutrition of the dog. Am J Physiol 130:365.

McKibbin JM, Thayer S, Stare FJ. 1944. Choline deficiency studies in dogs. J Lab Clin Med 29:1109.

Meir H, Yerganian GA. 1959. Spontaneous diabetes mellitus in the Chinese hamster (_Cricetulus grieseus_). I. Pathological findings. Proc Soc Exp Biol Med 100:810.

Mellanby M, Killick EM. 1926. A preliminary study of factors influencing calcification processes in the rabbit. Biochem J 20:902.

Mertz W. 1967. Biological role of chromium. Fed Proc 26:186.

Mertz W. 1969. Chromium occurrence and function in biological systems. Physiol Rev 49:163.

Mills CA. 1943. Heightened thiamine and choline requirements in tropical heat. Proc Soc Exp Biol Med 54:265-266.

Mills CA, Cottingham E, Taylor E. 1946. The effect of advancing age on dietary thiamine requirements. Arch Biochem 9:221.

Mirone L, Cerecedo LR. 1947. The beneficial effect of xanthopterin on lactation, and of biotin on reproduction and lactation, in mice maintained on highly purified diets. Arch Biochem 15:324.

Misler S, Klegar G. 1959. An automatic watering system for use in animal cages. Proc Animal Care Panel 9:67-70.

Monserrat AJ, Ghoshal AK, Hartroft WS, Prota EA. 1969. Lipoperoxidation in the pathogenesis of renal necrosis in choline-deficient rats. Am J Pathol 55:163.

Moore RA, Bittenger I, Miller ML, Hellman LM. 1942. Abortion in rabbits fed a vitamin K-deficient diet. Am J Obstet Gynecol 43:1007.

Moritz AR, Krenz C. 1930. The relation of the fat-soluble vitamins (A and D) to the development of experimental rickets in rabbits. J Nutr 2:257.

Morris HP, Lippincott SW. 1941. The effect of pantothenic acid on growth and maintenance of life in mice of the C3H strain. J Natl Cancer Inst 2:29.

Mosier J. 1973. Illinois Canine Symposium. University of Illinois.

Mrak EM. 1974. Chemical additives in our foods; a review and some calm observations. The Professional Nutritionist.

Munger BL, Lang CM. 1973. Spontaneous diabetes mellitus in guinea pigs. Lab Invest 29:685.

Nagler AL, Dettborn WD, Seifter E, Levenson SM. 1968. Tissue levels of acetyl choline and acetyl cholinesterase in weanling rats subjected to acute choline deficiency. J Nutr 94:13.

Naismith DJ. 1957-1958. Ascorbic acid requirements of the dog. Proc Nutr Soc 16-17.

NAS. 1965. National Academy of Sciences. A Report of the Committee on Standards, Institute of Laboratory Animal Resources. Standards for the breeding, care, and management of laboratory rabbits.

NAS. 1972. National Academy of Sciences. Nutrient requirements of dogs. Washington DC.

NAS. 1973. National Academy of Sciences. Toxicants occurring naturally in foods. 2nd Edition.

NAS. 1974. National Academy of Sciences. A Report of the Committee on Laboratory Animal Diseases. A guide to infectious diseases of guinea pigs, gerbils, hamsters, and rabbits.

NAS. 1978. National Academy of Sciences. A Report of the Committee on Laboratory Animal Diets. Control of diets in laboratory animal experimentation. ILAR News, Vol. XXI, Number 2.

Nelson AA, Fitzhugh OG, Calvery HO. 1943. Liver tumors following cirrhosis caused by selenium in rats. Cancer Res 3:230.

Nelson MM, Evans HM. 1955. Relationship of thiamine to reproduction in the rat. J Nutr 55:151.

Newberne PM. 1975. Influence on pharmacological experiments of chemicals and other factors in diets of laboratory animals. Fed Proc 34:209.

Newberne PM, Young VR, Gravice JF. 1969. Effects of caloric intake and infection on some aspects of protein metabolism in dogs. Br J Exp Pathol 50:172.

Nielsen E, Black A. 1944. Biotin and folic acid deficiencies in the mouse. J Nutr 28:203.

Nielson E, Elvehjem CA. 1941. Cure of spectacle eye condition in rats with biotin concentrate. Proc Soc Exp Biol Med 48:349.

NIH. 1978. National Institute of Health. Guide for the care of use of laboratory animals. NIH Publication No. 80-23.

O'Dell BL. 1960. Magnesium requirement and its relation to other dietary constituents. Fed Proc 19:648.

O'Dell BL, Hardwick BC, Reynolds G. 1961. Mineral deficiency in milk and congenital malformations in the rat. J Nutr 73:151.

O'Dell BL, Morris ER, Picket EE, Hogan AG. 1957. Diet composition and mineral balance in guinea pigs. J Nutr 63:65.

O'Dell BL, Morris ER, Regan WO. 1960. Magnesium requirements of guinea pigs and rats. Effect of calcium and phosphorus and symptoms of magnesium deficiency. J Nutr 70:103.

Olcese O, Pearson PB, Sparks P. 1949. Intestinal synthesis of niacin and the metabolic interrelationship of tryptophan and niacin in the rabbit. J Nutr 39:93.

Orent-Keiles E, Robinson A, McCollum EV. 1937. The effects of sodium deprivation on the animal organism. Am J Physiol 119:651.

Packer JT, Kraner K, Rose SD, Stuhlman RA, Nelson LR. 1970. Diabetes mellitus in *Mystromys albicaudatus*. Arch Pathol 89:410.

Parkes AS. 1946. Feeding and breeding of laboratory animals. I. Rat and mouse cubes, and cube containers. J Hyg 44:491-500.

Paterson JS. 1957. The role of an adequate diet in the production of healthy guinea-pigs. Lab Animals Bureau, MRC Laboratories. London. Collected Papers 5:55-59.

Peters RA. 1936. The biochemical lesion in vitamin B_1 deficiency. Application of modern biochemical analysis and its diagnosis. Lancet 1:1161.

Peterson FJ, Combs GF Jr, Holtzman JL, Mason RP. 1982. Effect of selenium and vitamin E deficiency on nitrofurantoin toxicity in the chick. J Nutr 112:1741-1746.

Pleasants JR. 1959. Rearing germfree cesarean-born rats, mice, and rabbits through weaning. Ann NY Acad Sci 78, Art. 1:116-126.

Poiley SM. 1972. Growth tables for 66 strains and stocks of laboratory animals. Lab Anim Sci 22:759-779.

Prentiss PG, Wolf AV, Eddy HA. 1959. Hydropenia in cat and dog. Ability of the cat to meet its water requirements solely from a diet of fish or meat. Am J Physiol 196:625-632.

Pudelkewicz C, Seufort J, Holman RT. 1968. Requirements of the female rat for linoleic and linolenic acid. J Nutr 94:138.

Purchase IFH. Symposium on Mycotoxins in Human Health. The Proceedings of a Symposium held in Preporia from 2nd to 4th September 1970 under the auspices of the South African Medical Research Council with the collaboration of the South African Council for Scientific and Industrial Research. The MacMillan Press Ltd, London.

Pye OF, Taylor CM, Fontanares PE. 1959. The effect of different levels of ascorbic acid in the diet of guinea pigs on health, reproduction, and survival. Fed Proc 18:Abstr No. 2132.

Quick AJ, Colletine GE Jr, Hussey CV. 1962. Vitamin K requirements of the growing pup. J Nutr 77:28.

Ralli EP, Dumm ME. 1953. Relation of pantothenic acid to adrenal cortical function. Vitam Horm 9:133.

Ramalingaswami V, Leach EH, Sriramachari, S. 1955. Ocular structure in vitamin A deficiency in the monkey. Q J Exp Physiol 40:337.

Ramalingaswami V, Sinclair HM. 1953. The relation of deficiencies of vitamin A and of essential fatty acids to folicular hyperkeratosis in the rat. Br J Dermatol 65:1.

Ranadive KJ. 1957. Contribution to discussion. Laboratory Animals Bureau, MRC Laboratories. London. Collected Papers 5:39-41.

Rauch H, Nutting WB. 1958. Biotin deficiency in the hamster. Experientia 14:382.

Rawls R. 1982. Fat substitute cuts patients' caloric intake. Chem Eng News. July 26, 1982.

Reber EF, Malhotra OP. 1961. Effects of feeding a vitamin K deficient ration containing irradiated beef to rats, dogs and cat. J Nutr 74:191.

Reid ME. 1954. Nutritional studies with the guinea pig. B-vitamins other than pantothenic acid. Proc Soc Exp Biol Med 85:547.

Reid ME. 1955. Nutritional studies with the guinea pig. III. Choline. J Nutr 56:215.

Reid ME. 1958. Guinea pig nutrition. Proc Animal Care Panel 8:23-33.

Reid ME. 1961. Nutritional studies with the guinea pig. VII. Niacin. J Nutr 75:279.

Reid ME. 1964. Nutritional Studies with the guinea pig. XI. Pyridoxine. Proc Soc Exp Biol Med 116:289.

Reid ME, Bieri JG. 1967. Nutritional studies with the guinea pig. VIII. Thiamine. Proc Soc Exp Biol Med 126:11.

Reid ME, Bieri JG, Plack A, Andrews EL. 1964. Nutritional studies with the guinea pig. X. Determination of the lineoleic acid requirement. J Nutr 82:401.

Reid ME, Briggs GM. 1954. Nutritional studies with the guinea pig. II. Pantothenic acid. J Nutr 52:507

Reid ME, Martin MG, Briggs GM. 1956. Nutritional studies with the guinea pig. IV. Folic acid. J Nutr 59:103.

Reid ME, Mickelson O. 1963. Nutritional studies with the guinea pigs. VIII. Effect of different proteins with and without amino acid supplements on growth. J Nutr 80:25.

Reid ME, Sallmann L Von. 1960. Nutritional studies with the guinea pigs. VI. Tryptophan with ample dietary niacin. J Nutr 70:329.

Richter CP. 1951. An ideal preparation for dissection of spinal, peripheral, and autonomic nerves of the rat. Science 112:20-21.

Robertson BT, Burns MJ. 1963. Zinc metabolism and the zinc-deficiency syndrome in the dog. Am J Vet Res 24(102):997.

Roebuck BD, Yager JD Jr., Longnecker DS, Wilpone SA. 1981. Promotion by unsaturated fat of azaserine-induced pancreatic carcinogenesis in the rat. Cancer Res 41:3961-3966.

Rogers AE, Newberne PM. 1971. Diet and aflatoxin B_1 toxicity in rats. Toxicol Appl Pharmacol 20-113.

Rollinson CL, Znig MG. 1981. Mineral nutrients. In: Encyclopedia of chemical technology. Vol. 15. Kirk-Othmer, John Wiley & Sons, N.Y.

Rose CS, Gyorgy P. 1950. Tocopherol requirements of rats by means of the hemolysis test. Proc Soc Exp Biol Med 74:411.

Routh JI, Houchin OB. 1942. Some nutritional requirements of the hamster. Fed Proc 1:191.

Rubin SH, DeRitter E. 1954. Vitamin A requirements of animal species. Vitam Horm 12:101.

Ruegamer WR, Elvehjem CA, Hart EB. 1946. Potassium deficiency in the dog. Proc Soc Exp Biol Med 61:234.

Sabrell WH, Onstott RH, Fraser HF, Datt FS. 1938. Nicotinic acid in the prevention of the black tongue of dogs. J Nutr 16:355.

Sadler PJ. 1982. Inorganic pharmacology. Chemistry in Britain 18(3),182-188.

Sauer F. 1961. Acetate metabolism in experimental ketosis of guinea pigs. Can J Biochem Physiol 39:739.

Sauer F. 1963. Fasting ketosis in the guinea pig. Ann NY Acad Sci 104:787.

Schaefer AE, McKibbin JM, Elvehjem CA. 1941. Importance of choline in synthetic rations for dogs. Proc Soc Exp Biol Med 47,365.

Scheid HEB, McBride BH, Schweigert BS. 1950. The vitamin B_{12} requirement of the Syrian hamster. Proc Soc Exp Biol Med 75:236.

Schoental R, Connors TA, eds. 1981. Dietary influences on cancer: traditional and modern. CRC Press, Inc., Boca Raton, Florida.

Schoental R, Joffe AZ, Yagen B. 1979. Cardiovascular lesions and various tumors found in rats given T-2 toxin, a trichothecene metabolite of fusarium. Cancer Res 39:2179-2189.

Schrader GA, Prickett CO, Salmon, WD. 1937. Symptomatology and pathology of potassium and magnesium deficiencies in the rat. J Nutr 14:85.

Schroeder HA. 1966. Chromium deficiency in rats: a syndrome simulating diabetes mellitus with retarded growth. J Nutr 88:439.

Schroeder HA. 1960. Relations between hardness of water and death rates from certain chronic and degenerative diseases in the United States. J Chron Dis 12:586.

Schroeder HA, Vinton WM Jr., Balassa JJ. 1963. Effect of chromium, cadmium and other trace metals on the growth and survival of mice. J Nutr 80:39.

Schwartz K. 1974. Recent dietary trace element research, exemplified by tin, fluorine, and silicon. Fed Proc 33:1748-1773.

Schwarz K. 1958. Dietary necrotic liver degeneration, an approach to the concept of the biochemical lesion in liver function. Brauer RW, ed. Symposium on liver function. Am Inst Biol Sci, Washington, DC. p. 509.

Schwarz K, Foltz CM. 1957. Selenium as an integral part of factor-3 against necrotic liver degeneration. J Am Chem Soc 79:3293.

Schwarz K, Foltz CM. 1960. Tocopherol sparing effect of S-amino acids. Fed Proc 19:421 (Abstr.).

Schweigert BS, McBride BH, Carlson HA. 1950. Effect of feeding polyoxyethylene monostearates on growth rate and gross pathology of weanling hamsters. Proc Soc Exp Biol Med 73:427.

Scudi J, Hamlin M. 1942. The effect of pantothenic acid deficiency on the blood lipids of the dog. J Nutr 24:273.

Selvey N, White PL, eds. 1980. Nutrition in the 1980's: constraints on our knowledge. Proceedings of the western hemisphere nutrition congress VL held in Los Angeles, California, August 10-14, 1980. Alan R. Liss, Inc., New York.

Sheng H, Huggins RA. 1971. Direct and indirect measurements of total body water in the growing beagle. Proc Soc Exp Biol Med 137:1093.

Shills ME, McCollum EV. 1943. Further studies on the symptoms of manganese deficiency in the rat and mouse. Nutrition 26:1.

Shimada T, Imai Y, Sato R. 1981. Covalent binding of polychlorinated biphenyls to proteins by reconstituted monooxygenase system containing cytochrome P-450. Chem-Biol Interactions, 38:29-44.

Shimatori N, Emerson GA, Evans HM. 1939. Role of vitamin E in the prevention of muscular dystrophy in guinea pigs reared on synthetic rations. Science 90:89.

Short DJ, Gammage LA. 1959. A new pelleted diet for rabbits and guinea pigs. J Animal Technicians Assoc 9:62-69.

Shwartzman G, Strauss L. 1949. Vitamin B6 deficiency in Syrian hamster. J Nutr 38:131.

Silber RH. 1944. Studies of pantothenic acid deficiency in dogs. J Nutr 27:425.

Silberberg M, Silberg R. 1955. Diet and life span. Physiol Rev 35:347.

Sisson JA, Plotz EJ. 1967. Effect of alloxan diabetes on maternal and fetal serum lipids in the rabbit. Exp Mol Pathol 6:274.

Slanetz CA, 1961. Problems in the care and nutrition of dogs and other laboratory animals. Proceedings of the animal care panel, 1st annual meeting. Vol. 11(3), Feb. 1961.

Slanetz CA, Fratta I, Crouse CW. 1957. Stock diets, special diet supplements and their effects on laboratory animals. Proc Animal Care Panel 7:203-211.

Smith SE, Ellis GH. 1947. Studies of the manganese requirement of rabbits. J Nutr 34:33.

Smith SE, Ellis GH. 1947. Copper deficiency in rabbits: achromotrichia, alopecia and dermatosis. Arch Biochem 15:81.

Smith SE, Medicott M. 1944. The blood picture of iron and copper deficiency anemias in the rat. Am J Physiol 141:354.

Smith DC, Proutt LM. 1944. Development of thiamine deficiency in the cat on a diet of raw fish. Proc Soc Exp Biol Med 56:1.

Smith RE, Reynolds IM. 1961. Leptospirosis in the hamster on diets containing various levels of riboflavin. Am J Vet Res 22:800.

Snell EE. 1953. Summary of known metabolic functions of nicotinic acid, riboflavin and vitamin B6. Physiol Rev 33:509-524.

Sober HA, Mannering GH, Cannon MD, et al. 1942. Nutrition of the guinea pig. J Nutr 24:503-514.

Srivastava U, Devi A, Sarker NK. 1965. Biochemical changes in progressive muscular dystrophy. III. Nucleic acid, phosphorus and creatinine metabolism in the muscle, liver and brain of rabbits maintained with a choline-deficient diet. J Nutr 86:298.

Stanley AJ, Hopps JC, Hellbaum AA. 1946. Observations on cobalt polycythemia. I. Studies on the peripheral blood of rats. Proc Soc Exp Biol Med 61:130.

Stellar E. 1959. Feeding behavior in the rodent. Am J Med Sci 237:404-405.

Street HH, Cowgill GR. 1939. Acute riboflavin deficiency in the dog. Am J Physiol 25:323.

Street HR, Cowgill GR, Zimmerman HM. 1941. Some observations of vitamin B_6 deficiency in the dog. J Nutr 21:275.

Stumpf WE, Sar M, Clark SA. 1982. Brain target sites for 1,25-dihydroxyvitamin D_3. Science 215:1403.

Swan KC, Salit PW. 1941. Lens opacities associated with experimental calcium deficiency. Am J Ophthalmol 24:611.

Talalay P, Takano GMV. 1952. Apparatus for tube-feeding rats. J Lab Clin Med 40:486-488.

Taylor S, Poulson E. 1956. Long term iodine deficiency in the rat. J Endocrinol 13:439.

Thomas TB, Emerson GA. 1945. Cytological findings in alloxan diabetes in the rabbit. Proc Trans Texas Acad Sci 28:89 (Abstr.).

Toreson WE, Lee JC, Grodsky GM. 1968. The histopathology of immune diabetes in the rabbit. Am J Pathol 52:1099.

Udall R. 1953. Nutrition of laboratory dogs. Proc Animal Care Panel, 4th Annual Meeting, Chicago, December 2-3, pp. 5-16.

U.S. Department of Health and Human Services, Public Health Service. 1981. Second Annual Report on Carcinogens, December 1981.

Vitale JJ, Hellerstein EE, Nakamura M, Lown B. 1961. Effects of magnesium deficient diet upon puppies. Circ Res 9:387.

Volk BW, Wellmann KF, Lazarus SS, Brancato P. 1969. Beta cell morphology and insulin immunoassay in long-term subdiabetic rabbits. Arch Pathol 88:413.

Voltin H, Schroeder HA. 1964. Famitial hypothalamic diabetes insipidus in rats (Brattleboro strain). Am J Physiol 206:425.

Wacker WEC, Vallee BL. 1958. Magnesium metabolism. N Engl J Med 259:431.

Warkany J, Schraffenberger E. 1944. Congenital malformations induced by maternal nutritional deficiency. VI. The preventive factor. J Nutr 27:475.

Warner RG, Davis RF, Sperling G, Loosli JK. 1953. Nutrition of small laboratory animals. Proc Animal Care Panel, 4th Ann Meeting, Chicago, December 2-3, pp. 17-73.

Weeks GG. 1957. The assessment of the diets of laboratory animals. Laboratory Animals Bureau, MRC Lab, Collected Papers 5:21-26.

Weisbuger JH. 1976. Bioassays and tests for chemical carcinogens. In: Chemical carcinogens, Charles E. Searle, ed. American Chemical Society.

Weise HF, Yamanaka W, Coon E, Barber S. 1966. Skin lipids of puppies as affected by kind and amount of dietary fat. J Nutr 89:113.

Wheatley VR, Sher DW. 1961. Studies of the lipids of dog skin. J Invest Dermatol 36:169.

Wigand G. 1959. Production of hypercholsterolemai and atherosclerosis in rabbits by feeding different fats without supplementary cholesterol. Acta Med Scand 166(Suppl. 351):1.

Wilgram GF, Hartroft WS, Best CH. 1954. Abnormal lipid in coronary arteries and aortic sclerosis in young rats fed a choline-deficient diet. Science 119:842.

Williams WL. 1960. Hepatic liposis and myocardial damage in mice fed choline-deficient or choline-supplemented diets. Yale J Biol Med 33:1.

Wilson JG, Warkany J. 1948. Malformations in the genital urinary tract induced by maternal vitamin A deficiency in the rat. Am J Anat 83:357.

Wilson JG, Warkany J. 1949. Aortic arch and cardiac anomalies in offspring of vitamin A deficient rats. Am J Anat 85:113.

Wilson RB. 1976. Diseases - deficiency and metabolic. CRC handbook on laboratory animals science. Vol 3. Melby GC Jr, Actman NH, eds.

Wilson RB, Newberne PM, Conner MW. 1973. An improved semisynthetic atherogenic diet for rabbits: Dietary fat carbohydrate interaction in atherogenesis. Arch Pathol 96:355.

Wilson RB, Newberne PM, Kula NS. 1974. Long-term protection by antioxidants against arterial sclerosis in choline-deficient rats. Exp Mol Pathol 21:118.

Witting LA, Horwitt MK. 1964. Effects of dietary selenium, methionine, fat level and tocopherol on rat growth. J Nutr 84:351.

Wolbach SB. 1946. Vitamin A deficiency and excess in relation to skeletal growth. Proc Inst Med Chic 16:118.

Wolbach SB, Howe PR. 1933. The incisor teeth of albino rats and guinea pigs in vitamin deficiency and repair. Am J Pathol 9:275.

Wolbach SB, Howe PR. 1928. Vitamin A deficiency in the guinea pig. Arch Pathol 5:239.

Wolf JM, Salter HP, Jr. 1931. Vitamin A deficiency in the albino mouse. J Nutr 4:185.

Worden AN. 1953. The feedings and husbandry of laboratory animals. Laboratory Animals Bureau, MRC Laboratories. Collected Papers 1:25-36.

Wostmann BS. 1959. Nutrition of the germfree mammal. Ann NY Acad Sci 78:175-182.

Wurtman RJ. 1982. Nutrients that modify brain function. Scientific American 246:50-59.

Wynder EL, Kline UE. 1965. The possible role of riboflavin deficiency in epithelial neoplasia. I. Epithelial changes of mice in simple deficiency. Cancer 18:167.

Yoshida A, Harper AE, Elvehjem CA. 1958. Effect of dietary level of fat and type of carbohydrate on growth and food intake. J Nutr 66:217.

50272-101

REPORT DOCUMENTATION PAGE

1. REPORT NO. EPA 560/6-83-005

2.

3. Recipient's Accession No.

4. Title and Subtitle
Nutritional Requirements and Contaminant Analysis of Laboratory Animal Feeds

5. Report Date
May 1984

6.

7. Author(s)
Bimal C. Pal, Robert H. Ross, Harry C. Milman (EPA)

8. Performing Organization Rept. No.
ORNL

9. Performing Organization Name and Address

Information Center Complex
P.O. Box X
Oak Ridge National Laboratory
Oak Ridge, TN 37831

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.
(C)
(G) DW930141-01-1

12. Sponsoring Organization Name and Address

Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street SW
Washington, DC 20460

13. Type of Report & Period Covered
Final

14.

15. Supplementary Notes

This report supersedes PB84111541.

16. Abstract (Limit: 200 words)

The primary objectives of this report are to present information concerning the nutritional requirements of several commonly used laboratory animal species (i.e., mouse, rat, hamster, guinea pig, rabbit, and dog) and to discuss various aspects of the problem of contamination of laboratory animal feeds. In addition, this document discusses the different types of laboratory animal diets (e.g., open vs closed formula), the ingredients used in these diets, the interaction of dietary components, and the public comments received respective to the EPA proposed guidelines for the nutrient composition of laboratory animal diets. Much of the data are presented in tabular form.

17. Document Analysis a. Descriptors

Laboratory animals
Diets
Nutrition
Vitamins
Preservatives
Contamination
Impurities
Pesticides
Nitroso compounds
Estrogens
Fungi
Molds
Aspergillus
Fusarium

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group Biology

18. Availability Statement
Release unlimited

19. Security Class (This Report)
Unclassified

20. Security Class (This Page)
Unclassified

21. No. of Pages
118

22. Price

(See ANSI-Z39.18)

See Instructions on Reverse

OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)
Department of Commerce